ARCHIVES OF PATHOLOGY

VOLUME 21 MAY 1936 NUMBER 5

EXPERIMENTAL PULMONARY EDEMA

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Pulmonary edema is seen at necropsy with great frequency. The presence of pulmonary edema is not difficult to interpret when it is associated with subacute nephritis, cardiac lesions, starvation or dietary deficiency. Edema may be produced experimentally by reducing markedly the protein content of the blood plasma, e. g., by plasmapheresis or by dietary deficiencies. Such edema is analogous to that occurring in nephrosis or after a period of starvation. The fluid in such cases has a low protein content and a low specific gravity. Apparently osmosis is the chief mechanism involved in the production of this type of edema.

Another type of edema occurs in cases of severe acute infection, intoxication and poisoning by drugs or after an extensive superficial burn, trauma or a surgical operation which involves much manipulation of the viscera. In such cases the fluid has a protein content closely approximating that of the blood plasma and a high specific gravity. We have found few reports of the experimental production of this type of pulmonary edema.

Edema of the lungs and of the gastro-intestinal mucosa was noted after experimental shock when death was somewhat delayed. The condition of the experimental animals when death was immediate was characterized by intense engorgement of the venules and capillaries of the lungs, serosa, mucosa, liver and kidneys and by evidence of capillary hemorrhages, but edema was not marked. It was further noted [1] that pulmonary edema was present regularly post mortem in human subjects when the shock syndrome had preceded death. Our studies led to the conclusion that *the shock syndrome arises from an uncompensated disparity between the volume of blood and the volume capacity of the vascular system.* Such a disparity frequently results from the presence of substances or conditions which cause the capillaries and venules to

Supported by the Martin Research Fund.

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1. Moon, V. H.: Ann. Int. Med. **8**:1633, 1935.

dilate and their walls to become permeable to blood plasma. Such dilatation increases the volume capacity of the vascular system. The escape of fluid through the permeable walls lowers the total volume of blood, causes hemoconcentration and produces edema. The fluid in such cases has a high specific gravity, and its composition resembles that of blood plasma. This shock syndrome occurs in varying degrees and with varying rapidity. These considerations suggested that pulmonary edema might be produced experimentally by inducing sublethal degrees of shock or degrees not immediately fatal.

Kennedy and one of us (Dr. Moon)[2] found that the introduction of fresh, finely chopped dog muscle into the peritoneal cavity of normal dogs caused acute shock. A dose of about 6 Gm. per kilogram of body weight caused hemoconcentration and death in ten hours. A quantity of gluteal muscle was excised with aseptic precautions from a freshly killed normal dog. It was finely ground in a sterilized meat chopper, and weighed amounts were suspended in four volumes of physiologic solution of sodium chloride. Varying quantities of this substance were introduced through a glass funnel into the peritoneal cavities of normal dogs under light ether anesthesia. Records were made of the pulse and respiratory rates and temperature and of the hemoglobin content and red blood cell count three times a day before and after this procedure.

It has been shown[3] that hemoconcentration is a regular feature in shock and that its use as an index avoids certain undesirable features associated with the taking of blood pressure records for the same purpose. The deep narcosis necessary for the making of kymographic records frequently produces shocklike manifestations, and it is impracticable to make records of blood pressure over a period of several days by the usual methods.

MUSCLE IMPLANTED IN THE PERITONEAL CAVITY

Three and five-tenths grams per kilogram of body weight of freshly ground dog muscle was implanted in the peritoneal cavity of a normal dog. Within twenty-four hours the number of red blood cells increased from 6,260,000 to 7,800,000 and the hemoglobin content from 14 to 18 Gm. per hundred cubic centimeters of blood. This indicated hemoconcentration of about 30 per cent. The respiratory rate rose from 28 to 48, the pulse rate from 112 to 136 and the temperature from 101.2 to 103 F. The animal was acutely ill; he shivered frequently, would not move about and refused food. The urine was scanty and contained albumin and visible blood. The animal died during the following night, probably thirty-six or forty hours after the operation.

The postmortem picture was characteristic of shock produced by various means. Since the same condition, with minor variations, was noted in each of the subsequent experiments, it will be detailed only once in this report. The

2. Moon, V. H., and Kennedy, P. J.: Arch. Path. **14**:360, 1932.
3. Moon, V. H., and Kennedy, P. J.: J. Lab. & Clin. Med. **19**:295, 1933.

blood, as seen in the veins, heart and other organs, was very dark and thick and had failed to clot. There was marked congestion of the pleura, pericardium and peritoneum. The lungs were intensely congested and heavy and did not collapse readily. There were petechial hemorrhages on the pleural surfaces. Blood-tinged frothy fluid escaped under pressure from the sectioned lung. The intestines were relaxed and distended, and their peritoneal surfaces were dusky red. The venules along the mesenteric attachment were markedly engorged. The mucosa was purplish red and swollen. The liver and kidneys were deeply congested. Blood oozed and dripped from the surfaces on section. The spleen was dry, firm and contracted. About 50 cc. of bloody fluid was present in the peritoneal cavity, but the other serous cavities contained no fluid.

Histologic examination showed marked diffuse congestion of the lungs. The venules were distended with closely packed corpuscles, and there were scattered capillary hemorrhages. The interstitial tissue of the alveolar walls was swollen so that the septums were two or three times the normal thickness. Many of the alveoli and bronchioles contained pink-staining fluid. The vessels in the liver and kidneys were engorged, and there were numerous capillary hemorrhages in the kidneys. The splenic pulp contained very few visible red blood cells. The capillaries in the adrenal glands were somewhat congested, but the adrenal cortex and medulla were otherwise normal.

Another dog was given 3 Gm. of muscle substance per kilogram of body weight. The hemoglobin value rose from 14 to 18 Gm. per hundred cubic centimeters of blood and the erythrocyte count from 6,110,000 to 7,900,000 within twenty-four hours, indicating hemoconcentration of about 30 per cent. The animal was acutely ill and refused food. The urine was scanty and contained blood and albumin. The hemoconcentration did not increase in the subsequent days. The dog died during the night following the fourth postoperative day.

The gross and microscopic observations were as described previously except that pulmonary edema was more marked and there was blood-tinged fluid in the serous cavities: 15 cc. in each pleural sac, 10 cc. in the pericardium and 25 cc. in the peritoneum. Microscopically the lungs showed marked engorgement and marked edema. About half the total alveolar space was filled with deeply pink-staining fluid. In many places there were numerous leukocytes in the fluid, both in the alveoli and in the bronchioles, indicating incipient pneumonia.

Three other dogs were given 2.5, 2 and 1.5 Gm., respectively, of muscle substance per kilogram of body weight intraperitoneally and were acutely ill for two or three days. Each of them showed hemoconcentration of about 25 per cent within twenty-four hours. There was a gradual return of the blood to its normal concentration, and all symptoms of illness disappeared within a week.

BURNS

In another experiment a dog was anesthetized, and about one third of the body surface was dipped momentarily into water at a temperature slightly below 100 C. (212 F.). On the following day the skin over the scalded area was reddened and somewhat swollen. The urine was scanty but contained no blood or albumin. The erythrocyte count rose from 5,000,000 to 6,700,000, and the hemoglobin value rose from 12 to 16 Gm. per hundred cubic centimeters of blood. Two days after the burn, moist bubbling râles could be heard throughout the chest. The scalded area healed without the loss of hair, the hemoconcentration subsided and the dog was apparently normal in seven days.

Another dog was scalded over slightly more than one third of the body surface for about five seconds. Hemoconcentration developed promptly and continued

with slight fluctuations for five days, after which it increased. After five days the temperature gradually rose from 101.2 to 104 F. on the eighth day; the pulse rate increased from 104 to 140 and the respiratory rate from 20 to 32 per minute. Moist bubbling râles could be heard throughout the chest, the urine was scanty and contained visible blood and the animal appeared very ill. He died eight days after the burn.

The gross picture was similar to that described by Bardeen,[4] Pack [5] and others in human beings following death from burns. It did not differ essentially from the condition seen after the peritoneal implantation of muscle substance. The pleura contained petechial hemorrhages. The lungs were deeply congested and edematous, and there were irregular scattered areas of partial consolidation. Microscopically the lungs were intensely congested throughout. There were

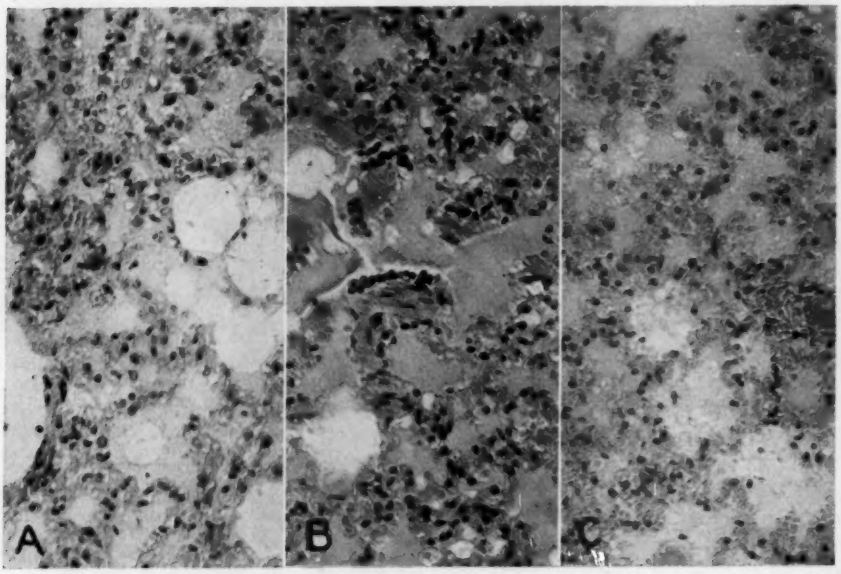

Fig. 1.—Photomicrographs showing appearance of lung tissue from dogs with experimental pulmonary edema: *A*, eight days after a superficial burn; *B*, thirty-six hours after intestinal obstruction; *C*, after repeated doses of histamine phosphate given subcutaneously. Marked edema, congestion and capillary hemorrhages are present in each. Magnification, × 250.

capillary hemorrhages, marked edema (fig. 1 *A*) and scattered areas of pneumonic consolidation. These features were similar to those seen in human beings with secondary pneumonia after a superficial burn.

INTESTINAL OBSTRUCTION

We are reporting elsewhere [6] the production of death by shock after intestinal obstruction in dogs. The symptoms and postmortem obser-

4. Bardeen, C. R.: J. Exper. Med. **2**:501, 1897.
5. Pack, G. T.: Arch. Path. **1**:767, 1926.
6. Moon, V. H., and Morgan, D. R.: Arch. Surg. **32**:776 (May) 1936.

vations in such cases did not differ essentially from those in cases in which shock was produced by other means. When death occurred twenty-four hours or more after experimental obstruction of the intestine, edema of the lungs was a regular feature. The following experiment is illustrative:

With the dog under light anesthesia a loop of the upper portion of the jejunum, approximately 10 inches (25 cm.) long, was tied with linen tape. Within twenty-four hours the hemoconcentration was over 30 per cent. The dog died approximately thirty-six hours after operation. At postmortem examination the obstructed loop was deep red and greatly distended. Both the lumen and the wall contained much blood, so that the entire loop looked as though involved by hemorrhagic infarction. There was marked congestion of all the serous surfaces, with scattered capillary hemorrhages in the pleura and peritoneum. About 250 cc. of blood-tinged fluid was present in the peritoneal cavity. There was no free fluid in the pleural or pericardial cavities. The lungs were congested and edematous. Blood-tinged frothy fluid flowed under pressure from the sectioned lung. The mucosa of the stomach and unobstructed portions of the intestine was markedly congested, and petechial hemorrhages were present. Congestion of the liver and kidneys was marked. The spleen was dry, firm and contracted. No changes were seen in the adrenals. Microscopic examination showed marked congestion of the capillaries and venules of the lungs and marked edema (fig. 1 *B*). Approximately half the total alveolar space was filled with pink-staining fluid. The perivascular lymph spaces were distended with fluid. The liver and kidneys showed parenchymatous degeneration and marked congestion. There were numerous capillary hemorrhages in the medulla of the kidney and marked congestion of the capillaries and moderate edema of the stomach and intestines. The spleen was anemic. The adrenals showed congestion of the capillaries but no other changes.

BILE AND BILE SALT

We have observed that acute pulmonary congestion and edema occur frequently after death from toxic jaundice. In those cases there was hemoconcentration, and death was due to circulatory failure. These observations suggested the use of bile and its salts for experimental purposes.

A dog was given daily intravenous injections of a 10 per cent solution of sodium glycocholate, 0.4 Gm. per kilogram of body weight. In six days the red blood cell count rose from 6,550,000 to 8,800,000 and the hemoglobin content from 14 to 19 Gm. per hundred cubic centimeters of blood. Beginning on the fifth day, a progressive decline in temperature and increased pulse and respiratory rates were noted. The postmortem observations were similar to those previously described, but the pulmonary edema was more extreme. There was a marked accumulation of fluid in the alveoli, and the perivascular lymph spaces were distended with fluid to a degree that we have never before seen either in clinical or in experimental edema (fig. 2).

Another dog, weighing 6.4 Kg., received seven intravenous doses of 2 Gm. each of sodium glycocholate in solution in thirty days. This produced hemoconcentration of 15 per cent, and a maximum of 25 per cent was reached during the last few days of the treatment. The animal died on the thirtieth day. There was

blood-tinged fluid in the pleural, pericardial and peritoneal cavities. The lungs were intensely congested. Bloody frothy fluid flowed from the bronchi without pressure. Its specific gravity was 1.020, as compared with that of the blood serum, 1.021. The lungs contained patchy areas of consolidation. Otherwise the picture was similar to that described previously. The lungs showed marked congestion of the capillaries with hemorrhages and edema. There were scattered areas of pneumonic consolidation.

Another dog died of acute circulatory failure three and one-half hours after a single injection of 0.4 Gm. per kilogram of sodium glycocholate in 10 per cent solution. Blood-tinged frothy fluid escaped from the nose and mouth before death. The red blood cell count rose from 4,600,000 to 7,240,000 and the hemoglobin content from 13 to 18 Gm. per hundred cubic centimeters of blood in three hours. The lungs were intensely congested and edematous. Blood-tinged frothy fluid

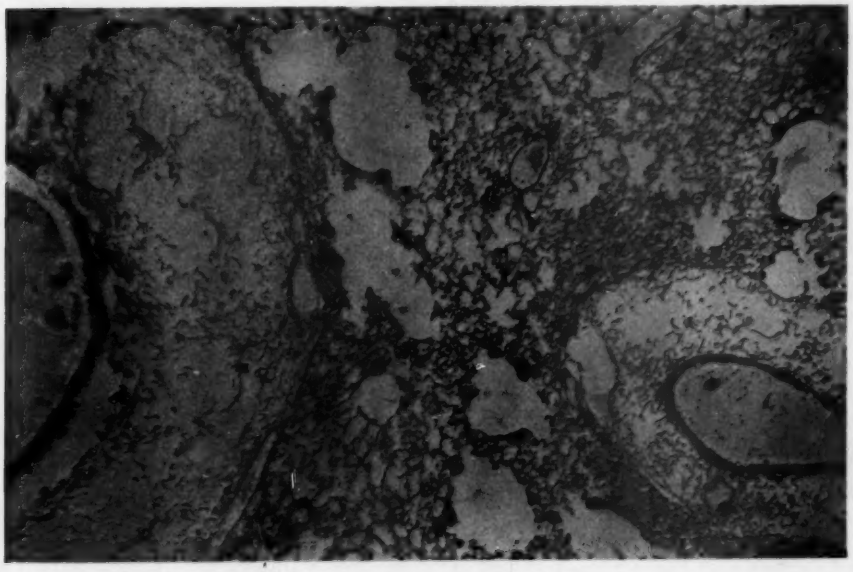

Fig. 2.—Low magnification of an area of lung after injections of sodium glycocholate. Most of the alveoli are filled with fluid. The remaining alveoli are emphysematous; several have been united into one space by rupture of their walls. The perivascular spaces are enormously distended with fluid.

flowed spontaneously from the trachea. This had a specific gravity of 1.022, while the specific gravity of the blood serum was 1.023. The viscera showed the same changes grossly and microscopically as described in the previous cases. Microscopically the lung substance was intensely congested. Marked edema, with distention of the perivascular lymph spaces and capillary hemorrhages, was present.

Apparently sodium glycocholate is more acutely injurious to vascular structures than is whole bile, as shown by the following experiment:

Quantities of normal bile had been saved from dogs used for physiologic and other experiments. This had been diluted with an equal volume of physiologic

solution of sodium chloride and had been filtered and sterilized by steam under pressure in the autoclave. A dog weighing 9.3 Kg. was given the following doses of diluted bile daily by intravenous injection: 80, 80, 100 and 120 cc. This amounted to a total of 190 cc. of undiluted sterile dog bile. The first three injections produced moderate evidence of illness and moderate hemoconcentration; after the last injection (60 cc. of bile) the dog became very sick. There were vomiting and apparent discomfort. The erythrocyte count rose from 5,250,000 to 6,100,000 and the hemoglobin value from 11 to 14 Gm. per hundred cubic centimeters of blood. The animal died within twenty-four hours.

The postmortem picture was similar to that already described. An additional feature was extensive hepatic necrosis. There were no petechial hemorrhages and no free fluid in the serous cavities. The lungs showed intense congestion and edema throughout. The specific gravity of the edema fluid was 1.024. Microscopically, marked diffuse congestion and edema of the lungs and of the bronchi were noted. Approximately 75 per cent of the total alveolar space was filled with fluid. In scattered areas there was moderate irregular leukocytic infiltration suggesting incipient pneumonia. There was extensive necrosis of the hepatic cells and of the renal epithelium.

Another dog was given two injections of 100 cc. each and one of 200 cc. of diluted bile intraperitoneally on successive days. This amounted to 200 cc. of undiluted whole bile. Small injections of morphine were given to alleviate pain. Vomiting, deeply bile-stained urine, failure of appetite and other evidences of acute illness were noted. The peripheral veins became collapsed, so that it was difficult to obtain blood, and the extremities grew cold. The red blood cell count increased from 4,640,000 to 5,970,000 and the hemoglobin content from 10 to 15 Gm. per hundred cubic centimeters of blood in three days. The animal died on the fourth day after the administration of the first injection.

The postmortem picture was the same as that previously described. There was 25 cc. of bile-tinged fluid in each pleural cavity and 150 cc. in the peritoneal cavity. The lungs showed marked congestion and edema (fig. 3*A*). No petechial hemorrhages were seen. The histologic picture was similar in all particulars to that in the previous case.

SODIUM PHENOBARBITAL

We have seen characteristic circulatory failure, with hemoconcentration and all the congestive features which accompany shock, develop in dogs occasionally after the intravenous injection of sodium phenobarbital in doses of 0.3 Gm. per kilogram of body weight. We have seen the congestive features characteristic of shock in human beings after poisoning with barbiturate compounds. It is known that some of these substances produce dilatation and permeability of the capillaries (Krogh,[7] Landis[8]). Accordingly, the following experiment was made:

A dog weighing 7.2 Kg. was given 0.3 Gm. of sodium phenobarbital per kilogram of body weight in solution by stomach tube twice daily. A total of 12.1 Gm. was given in three days. The dog was in a continuous state of profound narcosis

7. Krogh, A.: The Anatomy and Physiology of Capillaries, ed. 2, New Haven, Yale University Press, 1929.

8. Landis, E. M.: Physiol. Rev. **14**:408, 1934; Am. J. Physiol. **81**:124, 1927; **82**:217, 1927; **83**:528, 1928.

for four days and died in the night following the fourth day. The number of red blood cells increased from 5,150,000 to 6,950,000 and the hemoglobin content from 14 to 18 Gm. per hundred cubic centimeters of blood.

Postmortem examination showed the same changes as were noted in the previous cases. There were petechial hemorrhages in the pleura and peritoneum. The lungs were intensely congested and edematous, and blood-tinged frothy fluid flowed freely from the bronchi. The specific gravity of this fluid was 1.026, and that of the blood serum was 1.028. Microscopically the same intense congestion and marked edema were noted as in the previous cases. Approximately four fifths of the alveolar space of the lungs was filled with fluid, and there was much fluid in the bronchioles. There were scattered areas of leukocytic infiltration.

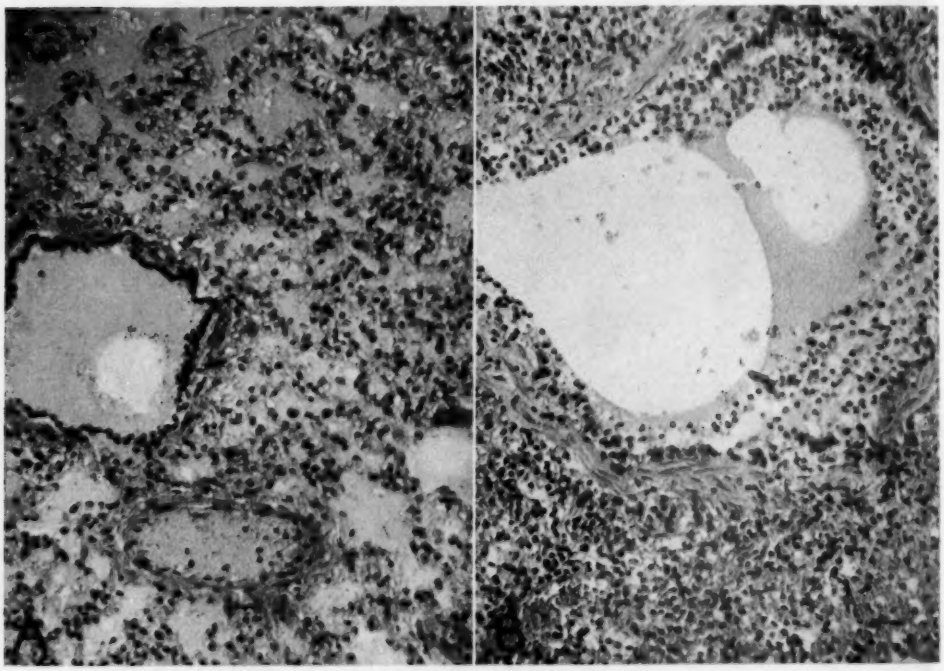

Fig. 3.—Microscopic appearance of lung tissue. *A*, pulmonary edema four days after the intraperitoneal introduction of sterile whole bile. An increased number of leukocytes is present. *B*, pulmonary edema, acute bronchitis and early pneumonia following prolonged narcosis due to sodium phenobarbital. Magnification, × 250.

Another dog, weighing 6 Kg., was given single doses of 0.3 of sodium phenobarbital per kilogram of body weight by stomach tube, with intervals of one or two days between doses. A total amount of 10.8 Gm. was given in ten days. As the effects of one dose wore off and the dog became active, he was given food and water before the next dose was given. The number of red blood cells increased from 5,300,000 to 7,300,000 and the hemoglobin content from 13 to 18 Gm. per hundred cubic centimeters of blood during that time. The animal died during the night following the tenth day after the first dose was administered.

Postmortem examination showed the same congestive changes as previously described. There were petechial hemorrhages in the visceral and parietal pleura, but no free fluid was present in the serous cavities. The lungs were intensely congested and edematous throughout, and there were irregular scattered areas of bronchopneumonia. The edema fluid had a specific gravity of 1.030. Microscopic examination showed marked engorgement of the pulmonary capillaries and venules, marked edema and an early stage of pneumonia, with irregular distribution (fig. 3*B*).

HISTAMINE

In another experiment a sterile solution of histamine phosphate (1:250) was given subcutaneously in doses of 7.5 mg. per kilogram of body weight. Two injections were given each day for three days, after which the dose was increased gradually until the original amount was doubled. These injections did not appear to cause local pain or discomfort, but areas of subcutaneous induration followed repeated injections. Evidences of systemic disturbances, such as weakness, inactivity, vomiting and oliguria, were prominent. The urine was dark and contained blood and albumin. The number of red blood cells rose from 5,330,000 to 6,460,000 and the hemoglobin content from 12 to 18 Gm. per hundred cubic centimeters of blood in eight days. The animal died on the morning of the ninth day.

The postmortem picture was the same as that noted in the preceding experiments. There were no petechial hemorrhages or free fluid in the serous cavities. The lungs were intensely congested, and there was moderate edema (fig. 1*C*). The adrenal glands were plump, rounded and larger than normal. Microscopically the lungs showed intense congestion, marked edema and capillary hemorrhages. Many of the alveoli and bronchioles were completely filled with albuminous fluid. There were local areas of leukocytic infiltration indicative of incipient pneumonia. The adrenal cortex was hyperplastic and about 50 per cent thicker than normal. The other tissues showed congestion and capillary hemorrhages, as in the previous experiments.

COMMENT

In 1893 Sherrington and Copeman [9] observed that animals subjected to prolonged experimental operative procedures showed increased respiratory and pulse rates and a decrease in the temperature and the blood pressure. Hemoconcentration and edema were present. The authors suggested the relationship of these phenomena to shock. Dale, Richards and Laidlaw [10] noted edema of the tissues following shock due to histamine. They attributed the edema to the increased permeability of the capillary walls and suggested that it had a significant bearing on the shock mechanism. Several authors have mentioned edema incidentally as a feature in experimental shock, but apparently they did not consider it a significant factor. Congestion and edema, especially of the lungs, were described regularly in military records of postmortem examinations after death from shock incidental to battle wounds. Frequently "wet autopsy" was used as a descriptive phrase in such cases

9. Sherrington, C. S., and Copeman, S. M.: J. Physiol. **14**:83, 1893.

10. Dale, H. H., and Richards, A. N.: J. Physiol. **52**:110, 1918. Dale, H. H., and Laidlaw, P. P.: ibid. **52**:355, 1919.

to denote a contrast to the "dry autopsy" performed after death from hemorrhage. But none of these observers commented on a significant relationship between the edema and the mechanism of shock.

Visible circulatory changes in the viscera, diffuse congestion and edema, particularly of the lungs, and widespread capillary hemorrhages are accepted as the characteristic pathologic changes due to burns. These observations were made (Long, 1840 [11]; Bardeen,[4] and Pack [5]) long before it was generally recognized that extensive superficial burns produce shock, before Moon and Kennedy called attention to the same visible changes in shock due to other causes and before the relationship of histamine to phenomena following burns had been suggested.

Löwit [11a] found pulmonary edema in dogs following poisoning with muscarine. Miller and Matthews [11b] produced pulmonary edema by injecting a large volume of 10 per cent aqueous solution of iodine U. S. P. intravenously. They referred to other reports of similar results and suggested that the edema resulted from vascular injury.

Manwaring and his associates [12] produced edema of the lungs in an experimental study of anaphylaxis. Perfusion of isolated organs of dogs, sensitized to horse serum, with fluid containing a small amount of the antigen led to marked edema of the lungs and intestines, accompanied with vasoconstriction of the arteries. Similar perfusion in nonsensitized dogs produced no such effects. The author stated the belief that in these experiments anaphylaxis affected specifically the permeability of the capillary walls.

The subcutaneous injection of para-phenylene-di-amine produces edema (Hanzlik and Tainter,[13] Gibbs [14] and others), which appears first about the head and neck and later in other parts. Sollmann [15] stated that the effects of para-phenylene-di-amine agree in principle with those of histamine, in that the drug produces contraction of smooth muscle, constriction of the bronchi and increased permeability of the capillaries, with resulting inflammatory and edematous phenomena. No explanation was offered for the specific location of the edema or for the observation that an isomer of the drug, meta-phenylene-di-amine, produces edema with hydrothorax. Landis [8] recently reviewed the literature on permeability of the capillaries. He stated that his own obser-

11. Long, J.: London M. Gaz. **1**:743, 1840.

11a. Löwit, M.: Beitr. z. path. Anat. u. z. allg. Path. **14**:401, 1893.

11b. Miller, J. L., and Matthews, S. A.: Arch. Int. Med. **4**:356, 1909.

12. Manwaring, W. H.; Chilcote, R. C., and Hosepian, V. W.: J. Immunol. **8**:233, 1923.

13. Hanzlik, P. J., and Tainter, M. L.: J. Lab. & Clin. Med. **9**:166, 1923.

14. Gibbs, O. S.: J. Pharmacol. & Exper. Therap. **20**:221, 1922.

15. Sollmann, T.: A Manual of Pharmacology, ed. 4, Philadelphia, W. B. Saunders Company, 1932, p. 469.

vations were in agreement with those of Dale, Krogh, Lewis and others. He summarized these with the generalization that any substance or condition which causes injury to the endothelium of the capillaries renders it abnormally permeable to plasma fluids. That generalization is exemplified in each of our observations on the occurrence of the shock syndrome, its experimental production and the visible circulatory changes which accompany it. The walls of the capillaries and venules are abnormally permeable, as shown by the visible leakage of serum and erythrocytes through them and by the escape of colloidal dyes [6] which are retained by normal capillaries. Also the capillaries and venules are markedly dilated and engorged, as seen grossly and microscopically. The combination of vascular dilatation and the escape of fluid from the blood results in a disparity between the blood volume and the volume capacity of the vascular system. Such a disparity manifests itself in the shock syndrome.

Best, Dale, Dudley and Thorpe [16] stated that the depressor effect of various tissue extracts is due to their content of histamine and of choline. Thorpe [17] made a study of the amount of histamine present in normal muscle. From 35 Kg. of muscle he secured in the crude extract the equivalent of 140 mg. of histamine. Much of this was lost in the subsequent chemical procedures, so that only 3.3 mg. of histamine was finally recovered. He gave as his conclusion, though admittedly without absolute proof, that the depressor effect of the alcoholic extract of muscle is due to its histamine content.

In several of our experiments 20 Gm. of fresh dog muscle produced fatal shock when introduced into the peritoneal cavity of a dog weighing from 5 to 7 Kg. The fatal dose of histamine phosphate given by intravenous injection to dogs is about 30 mg. per kilogram of body weight (Sollmann). These dogs then would have required from 150 to 200 mg. to produce fatal shock. Such amounts are far above the maximum quantity which could be derived from 20 Gm. of muscle. Obviously some substance other than histamine must be responsible for the circulatory effects produced in these experiments. Vaughan [18] showed that products of protein cleavage, produced either by chemical or by enzymatic action, are highly toxic. The injection of such products into animals, regardless of the character or source of the protein, was followed by an increase in the pulse and respiratory rates, marked variations in temperature and other evidences of severe illness. In some experiments the temperature was markedly elevated; in others a lower-

16. Best, C. H.; Dale, H. H.; Dudley, H. W., and Thorpe, W. V.: J. Physiol. **62**:397, 1927.

17. Thorpe, W. V.: Biochem. J. **22**:94, 1928.

18. Vaughan, V. C.; Vaughan, V. C., Jr., and Vaughan, J. W.: Protein Split Products in Relation to Immunity and Disease, Philadelphia, Lea & Febiger, 1913.

ing of the temperature preceded death. These investigations were made (1906 to 1912) when little was known regarding shock. No observations on the pressure or the concentration of the blood were made. Death apparently was caused by circulatory failure. The postmortem observations (Morse [19]) coincided closely with those made regularly in our study of shock. It seems probable that products of protein cleavage resulting from the autolysis of muscle and of other tissues are injurious to the capillary endothelium and that shock following extensive injury to muscles or following the experimental implantation of muscle substance is due largely to the effects of these products.

In previous articles [20] have been reported the congestive features, including pulmonary edema, characteristic of the shock syndrome as seen after death from surgical shock, burns, metabolic intoxication, severe abdominal conditions, such as intestinal obstruction, poisoning with sedatives and other drugs, and severe acute infection. In each of these conditions the shock syndrome had preceded death, and in each case hemoconcentration was a prominent feature. Several of these conditions have been approximated in the experiments here reported. Shock of varying degrees has followed the introduction of muscle substance intraperitoneally, burns, intestinal obstruction, the intravenous or intraperitoneal administration of bile and bile salts, poisoning due to barbital or the subcutaneous administration of histamine. In each instance marked hemoconcentration developed, and the tissue changes characteristic of shock were present in marked degree, as shown by gross and microscopic examinations. When death was not immediate, marked pulmonary edema developed.

It has been emphasized [1] that shock occurs in varying degrees and with varying rapidity, that maximal degrees lead to death from circulatory failure and that lesser degrees may be followed by recovery or by the development of terminal pneumonia. One of us (Moon [21]) reported observations and interpretations regarding this form of pneumonia in man. When the pulmonary circulation is impaired and when the spaces in the lungs are filled with edema fluid, the conditions are particularly suitable for the development of pneumonia. These observations have been substantiated in the experiments described. Sublethal degrees of shock were produced, followed by spontaneous recovery. Shock with subsequent death was accompanied with marked pulmonary edema, and when death occurred after an interval of several days pneumonia of a secondary type and with an irregular distribution developed.

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19. Morse, quoted by Vaughan,[18] p. 388.

20. Moon.[1] Moon and Kennedy.[2, 3]

21. Moon, V. H.: Am. J. Path. **9**:899, 1933.

The congestive features which accompany shock resemble closely those of passive congestion and are generally mistaken for passive congestion in necropsy examinations. These points of similarity and the differentiation of shock from passive congestion will be discussed in a subsequent report.

CONCLUSIONS

A type of pulmonary edema which occurs frequently in man has been reproduced experimentally by various simple means: the introduction of muscle substance into the peritoneal cavity, burns, intestinal obstruction and the injection of sodium glycocholate, bile, sodium phenobarbital or histamine. Doubtless other agents injurious to the capillary endothelium would also produce edema.

Pulmonary edema of this type results from increased vascular permeability and is accompanied with hemoconcentration and circulatory inefficiency. The mechanism of such edema is integral with that of the shock syndrome.

When fatal shock develops rapidly, congestive changes with slight edema are characteristic of the postmortem picture. When death is somewhat delayed, marked edema is seen. A common type of terminal pneumonia develops if neither death nor recovery occurs soon.

MULTIPLE MYELOMA OF HEMOCYTOBLASTIC TYPE

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The origin and nature of multiple myeloma and its place in the oncologic system have been much discussed. In a histogenetic classification one can distinguish (1) erythroblastoma, (2) myelocytoma, (3) plasmocytoma and (4) lymphocytoma. Usually tumors of the last-mentioned category are the most malignant, the group including neoplasms of varying structure, especially the so-called lymphosarcomas of the bone marrow.

The main problem has become increasingly cytologic, and the following observation may contribute to the discussion of the histogenesis.

REPORT OF A CASE

A white man, aged 52, who had always been healthy, fell and broke a rib in March 1935. Two months later he noticed a lump over the site of the injury (ninth rib on the right side), the pain at the costocartilaginous junction increasing steadily and radiating toward the vertebrae.

Clinical examination (service of Dr. N. H. Gosse, the Victoria General Hospital, Halifax) showed nothing abnormal in the lungs and heart and the nervous and gastro-intestinal systems. The results of urinalysis were normal. Moderately severe secondary anemia was present. The blood count was as follows: hemoglobin concentration 55 per cent, red cells 3,560,000, and white cells 4,600, per cubic millimeter, abnormal cells none, polymorphonuclears 66 per cent, lymphocytes 31 per cent and large mononuclears 3 per cent. Achromia, anisocytosis and poikilocytosis were present. The test for occult blood gave negative results. The reactions to the Kahn and Hinton tests were negative.

A roentgenogram showed that about one sixth of the ninth rib was absorbed, owing to osteoporosis. Bence-Jones' albumosuria was not shown.

After progressive mental deterioration, associated with vomiting and emaciation and moderate increase in the temperature and pulse rate, the patient died, seven months after the fracture of the rib and five months after the onset of a noticeable swelling.

Autopsy.—Gross Examination: The body was much emaciated. An oval swelling, measuring 6 inches (15 cm.) in length by 3 inches (7.5 cm.) in width and 1½ inches (3.8 cm.) in depth, was situated between the eighth and the tenth rib in the right posterior axillary line, extending toward and involving the corresponding dorsal vertebrae. When the ninth rib was opened the bony tissue was

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observed to be destroyed and replaced by a hemorrhagic soft pinkish-gray tumor. Another tumor about the size of a pigeon's egg, situated 3 inches (7.5 cm.) from the costocartilaginous junction, involving the eighth rib and protruding into the pleural sac, was of the same character. On horizontal section multiple pinkish-gray tumors, the largest measuring about ½ inch (1.3 cm.) in diameter, were visible in the body of the ninth rib, and also nodules the size of a small pea were present in the bodies of the sixth to the twelfth dorsal vertebra inclusive. The marrow of the sternum, ribs and vertebrae otherwise was the color of raspberry jelly, the bony substance being softened.

In the right pleural cavity no free fluid was present, but beneath the swelling in the ninth rib multiple pea-sized paler tumor nodules in the pleura were observed, extending to and involving the diaphragm.

The lymph nodes along the descending part of the aorta were moderately enlarged and evidently infiltrated by tumor.

The marrow of the right femur was the seat of a regenerative erythroblastic and lymphoid reaction. The skull and other bones were not involved, and no metastases in the other organs were noted.

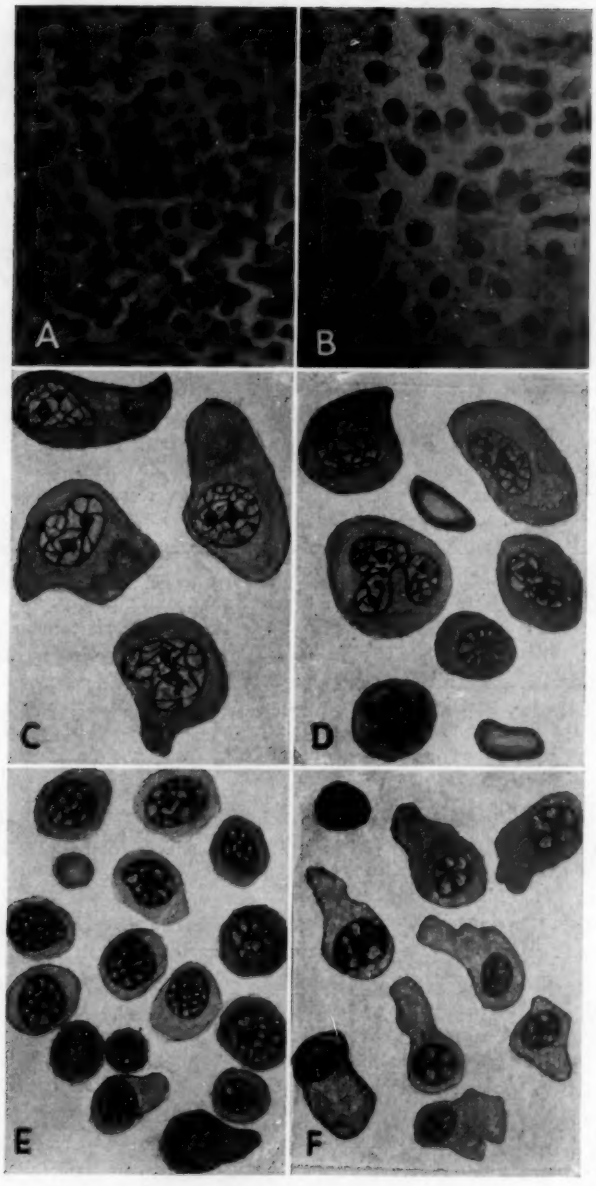
Other pathologic observations were chronic bronchitis, small calcified healed fibroid tubercles in the apexes of both lungs, a healed Ghon lesion in the middle of the right lower lobe posteriorly, terminal hypostatic pneumonia with edema, small hemorrhagic infarction in the left lower lobe, hemorrhagic erosions of the stomach, atrophy of the myocardium, slight nodular atheroma of the aorta, and fibrosis and decrease in the size of the prostate, with no evidence of neoplasm, and moderate generalized edema of the brain.

For histologic examination blocks from representative areas of the various tumors were fixed in a dilute solution of formaldehyde U. S. P. (1:10) or a mixture of Zenker's fluid and a dilute solution of formaldehyde. Sections from the eighth and ninth ribs, the bodies of the sixth to the twelfth dorsal vertebra, the diaphragm, the marrow of the right femur and lymph nodes were stained with the hematoxylin and eosin, with hematoxylin and Van Gieson's stain, with hematoxylin, azure II and eosin and with borax and methylene blue.

Microscopic Examination: The analysis of the organs failed to show anything of interest but excluded the presence of nephritis.

With low and medium power magnification the various areas of tumor from the ribs and vertebrae were of uniform appearance. Masses of round cells occurred with scanty connective tissue fibers between them, and in some areas small hemorrhages and capillaries filled with blood were surrounded by small and medium-sized round cells with irregular cytoplasm (fig. *A* and *B*). With the hematoxylin-azure II-eosin stain bluish areas predominated, but other parts appeared reddish, thus indicating also the presence of acidophilic cells.

Study of the finer structure of the individual cells with the oil immersion lens revealed (fig. *D* and *E*) a dense accumulation of large and small round or spheroid cells with light and amblychromatic nuclei, containing much chromatin with a triangular or square arrangement. The cytoplasm was homogeneous and variable in color, being bluish or grayish and sometimes pinkish, according to the amount of acidophilic substance. These cells were polychromatophilic erythroblasts in various stages of differentiation. Furthermore, proerythroblasts were present, the nuclei of which still showed purple nucleoli. Other cells, as small as ordinary erythrocytes, had round nuclei which stained intensely dark blue and contained thick particles of chromatin showing a checkered pattern but no nucleoli and a narrow round band of homogeneous cytoplasm, which stained reddish with eosin. These were oxidophilic normoblasts. Here and there erythrocytes were noted.



(*A*) Photomicrograph of the tumor with medium magnification; (*B*) photomicrograph of the tumor with higher magnification; (*C*) drawing showing a group of hemocytoblasts; (*D*) drawing of three cells, showing transitional stages between the hemocytoblast and the proerythroblast, and of a megakaryocyte, a polychromatophilic erythroblast, showing mitosis, a normoblast and two red cells; (*E*) drawing of a group of polychromatophilic erythroblasts, showing an increasing amount of hemoglobin, proerythroblasts and normoblasts and one red cell, and (*F*) drawing of tumor cells of the plasma cell type and one degenerated lymphocyte.

All the drawings (*C* to *F*) were made with the same magnification with the camera lucida at the level of the microscope stage, oil immersion lens 1/12 and ocular 18 ×. Hematoxylin-azure II-eosin stain.

Scattered throughout the sections a few typical megakaryocytes were seen, with light cytoplasm and a large round or irregular nucleus, consisting of vesicular segments of different sizes united by narrow membranous bridges; the nuclei were filled with chromatin, but no nucleoli, in the strict sense, were visible.

In addition, a group of nongranular basophilic lymphoid cells was noticeable, namely, hemocytoblasts; i. e., the nuclei of these cells, each containing two or three purple nucleoli, were extremely large and eccentric, with a thin, retracted membrane and a fine threadlike network of chromatin, becoming dense at the junctions. The cytoplasm of these cells was relatively abundant, homogeneous and apparently basophilic and occasionally showed small vacuoles. Mitotic figures were observed everywhere (fig. *C*).

In the acidophilic areas but still more prominently in the basophilic zones were small and medium-sized true lymphoid cells with a very thick nuclear membrane; the chromatin had a broad based and triangular outline and was pointed toward the centrally situated nucleoli, giving the appearance of a cartwheel. The cytoplasm was abundant, with irregular edges and sometimes small purple enclosures (fig. *F*). These cells were plasma cells. They stained selectively with borax and methylene blue and constituted the main bulk of the tumor cells in the majority of the areas examined.

Finally, other polyblastic or kidney-shaped cells were detected, with large irregular nuclei, irregular particles of chromatin and small, hardly visible nucleoli; the cytoplasm was light and abundant and contained greenish-brown enclosures. These cells were reticular histiocytes, showing phagocytosis of blood pigment. Other cells had broad cytoplasm, and the nucleus showed a fine chromatin reticulum and thin membrane and from two to three nucleoli. This cell probably represents a transition between the histiocyte and the fibrocyte. Here and there cells of the undifferentiated reticulum were noted, or transitional stages between them and hemocytoblasts on the one hand or hemocytoblasts and proerythroblasts on the other.

Smears made from the marrow of the femur revealed normoblasts, erythroblasts of the orthochromatic and polychromatic types, associated with poikilocytosis, myelocytes and promyelocytes of the neutrophilic, basophilic and eosinophilic groups and, furthermore, many hemocytoblasts. Cells of the lymphoid group were scarce, and there were few monocytes, megakaryocytes, endothelial cells and reticulum cells. This points to a very early stage of transition between reactive hyperplasia of the cells of the bone marrow and true tumor growth.

COMMENT

The tumor thus consisted of a large variety of blood cells in various stages of differentiation. First, cells of the red cell series were present in all grades of maturation: proerythroblasts, polychromatophilic erythroblasts, normoblasts and erythrocytes. Small and medium-sized lymphocytes were seen, with conversion into cells of the plasma cell type. Megakaryocytes were noted. In addition, a considerable number of hematocytoblasts were detected, i. e., the omnipotent free lymphoid hematopoietic cell, originating from the undifferentiated mesenchymal cell, and transitional stages between it and the proerythroblast. Such observations prove that the tumor, although appearing uniform under low power magnification, owing to the variety of blood cells

present cannot be considered as simple sarcoma, lymphosarcoma of the bone marrow or endothelial myeloma (Ewing's tumor). As no other primary tumor was observed, one is dealing with a primary growth of the bone marrow itself. The only question which arises concerns the histogenesis.

Ewing [1] suggested the origin of the myeloma cells from the adventitial cells of the vessels of the marrow. Boyd [2] expressed the belief that the variety of cells represents merely "variations of the same cell." Kaufmann [3] separated from multiple myeloma a group of malignant lymphosarcomas of the bone marrow in which the cells are larger than plasma cells but giant cells, reticular matrix and metastases are observed. This tumor corresponds to Ewing's group of more malignant myelomas.

Apparently the tumor in the present case belongs to the same category. The high clinical malignancy is supported by the presence of many relatively immature cells. Geschickter and Copeland [4] collected four hundred and twenty-five cases from the literature and expressed the belief that the type cell of the myeloma must be an "as yet unrecognized specific marrow cell." In 1933 Scott, Stanton and Oliver [5] supplemented this collection with thirty additional cases, five being those of their own observation. They suggested the derivation of the tumor plasma cell from the reticulum cell, while Williams [6] favored the osteoblast as the mother cell.

On the basis of a critical review of the microscopic appearance in the reported cases, we note that some authors have described a combination of plasma cells and erythroblasts or even of myelocytes and lymphoid cells, and, recently, (Zäh [7]) of myeloblasts and erythroblasts, within the same tumor.

In consideration of these facts it is only logical to assume that all the different cell types may have a common source. This progenitor cell, proliferating under tumor conditions, retains all its hematopoietic potentialities so that differentiation may take place in all directions and a most varied cytologic structure result.

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1. Ewing, James: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928.

2. Boyd, W.: Surgical Pathology, Philadelphia, W. B. Saunders Company, 1933, p. 729.

3. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie für Studierende und Aerzte, Berlin, G. Reimer, 1922.

4. Geschickter, C. E., and Copeland, M.: Arch. Surg. **16**:807, 1928.

5. Scott, E.; Stanton, F. M., and Oliver, M.: Am. J. Cancer **17**:682, 1933.

6. Williams, H. W.: Am. J. Cancer **16**:540, 1932.

7. Zäh, K.: Virchows Arch. f. path. Anat. **283**:310, 1932.

The more recent descriptive and experimental work (Maximow,[8] Downey,[9] Dominici,[10] Bloom [11] and Silberberg [12]) has elucidated more and more clearly that it is the hemocytoblast which keeps its hematopoietic function throughout life. In the present case the hemocytoblast was definitely seen in all the tumor eruptions. Therefore, we have good reason to conclude that myeloma is a systemic disease of the hematopoietic tissues of the bone marrow, the source being the hemocytoblast, which we assume to be the specific "as yet unrecognized marrow cell" of Geschickter and Copeland. Either these cells may undergo differentiation in one determined direction or mixed types with different cell groups may result. This conception also aids in answering the question whether multiple growths are of independent origin or are metastatic. With the hemocytoblast as the progenitor cell, the former hypothesis could well be explained.

SUMMARY

In a case of myeloma consisting of a mixture of lymphocytes, plasma cells, immature red cells and megakaryocytes, hemocytoblasts were observed. The various cells in this particular case were derived from proliferation of hemocytoblasts, which retained their hematopoietic potentialities. The conclusion is drawn that probably all myelomas originate from this progenitor cell, the varied types being produced by differentiation in one direction or another.

8. Maximow, A., in von Möllendorff, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, p. 232.

9. Downey, H. A.: Arch. Int. Med. **33**:301, 1921; Haematologica **3**:431, 1922; Folia haemat. **34**:65, 1927.

10. Dominici, H.: Arch. de méd. expér. et d'anat. path. **14**:1, 1902; Arch. d'anat. micr. **17**:1, 1921.

11. Bloom, W., in Hirschfeld, H., and Hittmair, A.: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwarzenberg, 1933, vol. 1, p. 1179.

12. Silberberg, M., in Hirschfeld, H., and Hittmair, A.: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwarzenberg, 1933, vol. 1, p. 1319.

RELATION OF GLIOMA OF THE LEPTOMENINGES TO NEUROGLIA NESTS

REPORT OF A CASE OF ASTROCYTOMA OF THE LEPTOMENINGES

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Considerable evidence has been published in recent years to support the derivation of the leptomeninges from the neural crest.[1] Some authors, especially among the French, have referred, therefore, to all tumors arising from them as gliomas.[2] These authors classified the tumors with neurofibromas, which have been referred to as peripheral gliomas.[3] The common tumors of the leptomeninges, usually called meningiomas, have little in common with the glial tumors arising within the brain substance. It is the purpose of this paper to call attention to certain unusual tumors of the leptomeninges which are identical in histologic appearance with the gliomas of the brain substance and to consider the possible relationship of such tumors to neuroglia nests in the meninges. These tumors are not related to so-called gliomatosis of the meninges, in which there is seeding of the meninges from a glioma arising in the brain substance,[4] or to the sarcomatous tumors of the leptomeninges.[5]

REPORT OF CASE

Clinical History.—A white man, aged 39, was admitted to the hospital because of convulsive seizures. During the preceding seven months he had noted slight drooping of the left eyelid and had suffered loss of memory for recent events. For three months he experienced difficulty in expressing words and ideas already formulated in his mind and was unable to concentrate. Two weeks before admission to the hospital he had a convulsive seizure lasting ten minutes. He had a similar attack four days before admission and five attacks on the first day in the hospital. Each attack was manifested by increased activity followed by a cry. The pupils were dilated, and the eyes rolled upward, the head being drawn back-

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1. (*a*) Harvey, S., and Burr, H.: Arch. Neurol. & Psychiat. **15**:545, 1926. (*b*) Oberling, C.: Bull. Assoc. franç. p. l'étude du cancer **11**:365, 1922.

2. Roussy, G., and Cornil, L.: Ann. d'anat. path. **2**:63, 1925. Martin, J.-F.; Dechaume, J., and Puig, R.: Ann. d'anat. path. **5**:277, 1928. Oberling.[1b]

3. Lhermitte, J., and Leroux, R.: Bull. Assoc. franç. p. l'étude du cancer **9**:112, 1920. Masson, P.: Am. J. Path. **8**:367 and 389, 1932.

4. Brannan, D.: Am. J. Path. **2**:123, 1926.

5. Bailey, P.: Arch. Surg. **18**:1359, 1929.

ward and to the right. Clonus began in the right arm, spreading to the left arm and legs, and lasted about one minute. A period of apnea followed for forty seconds, during which the patient became deeply cyanotic. The clonic motions ceased first in the legs and then in the right arm and persisted longest in the left arm. Deep, stertorous respirations then began, and the cyanosis disappeared. Complete relaxation of the musculature, cold perspiration and unconsciousness supervened. During the attacks there were incontinence of urine and relaxation of the sphincter of the rectum. At about this time persistent headaches developed in the left frontoparietal region, which spread to involve the left side of the occiput. Examination, including neurologic and ophthalmoscopic observations, roentgenograms of the skull, the Wassermann test and lumbar puncture, showed nothing abnormal. Permission for encephalography was refused. The clinical diagnosis was suspected tumor of the brain.

After the patient was discharged from the hospital, one month later, the headaches improved, but the inability to concentrate, forgetfulness and aphasia persisted. Three months later the headaches again became very severe, and there was persistent tenderness in the left frontoparietal region. Uncontrollable projectile vomiting developed. A white circular light appeared in the field of vision of both eyes.

The patient was readmitted to the hospital after four months because of a convulsive seizure similar to the previous ones. Examination showed marked motor aphasia, bilateral papilledema with fresh hemorrhages along the temporal border of the left disk, engorgement of the retinal vessels, slight bilateral exophthalmos, tenderness in the left temporal region and weakness of the musculature of the right side of the face, with flattening of the right nasolabial fold. The tendon reflexes of the upper and lower extremities were hyperactive. The Babinski, Oppenheim and Gordon reflexes were of the plantar type.

Operation.—With the area under procaine hydrochloride anesthesia, a large bone flap was reflected in the left frontoparietal region. In the dura numerous blood vessels, one of which was very large, were centered over a tumor at the posterior border of the frontal lobe, having a broad attachment to the dura mater at this point. The dura was incised in circular fashion about the adherent tumor. The tumor could then be rocked out of its position and the brain pushed away. The operator could observe no connection between the tumor and the brain itself. The blood supply of the adjacent leptomeninges was more abundant than usual, and numerous blood vessels entered the tumor from that region as well as from the dura. No vessels entered the tumor from the substance of the brain, and no bleeding was encountered in the bed of the tumor as it was enucleated. A large depression in the brain substance was left, which was partially filled by expansion of the underlying cerebral cortex. Because of the superficial location, the encapsulated appearance, the absence of adherence to the cortex and the obvious foothold on the dura, as well as the nature of the blood supply, the operative diagnosis was meningioma.

Postoperative Course.—After operation, the patient did well and was discharged in twenty-two days, free from any complaint. Two months later he noted the return of difficulty in concentration, irritability, aphasia and vomiting. Examination showed bulging at the site of decompression, choking of the optic disks and evidence of involvement of the pyramidal tracts. Three roentgen treatments with doses of 300 roentgens each were given in the right and left frontal regions, and two treatments with 300 roentgens each, in the region of the cerebellum and the cervical and thoracic portions of the spine. The patient's condition was essentially unchanged. Eleven months after operation he was confined to bed. He presented complete motor aphasia and was able to speak only in monosyllables. There

was tremendous bulging at the site of the subtemporal decompression. Convulsions occurred occasionally, and the right arm was paralyzed. The patient died thirteen and one-half months after operation. The terminal syndrome was one of increasing intracranial pressure, right hemiplegia and bronchopneumonia. Permission for autopsy could not be obtained.

Macroscopic Description of the Specimen.—The specimen consisted of an ovoid tumor, measuring 7.5 by 6 by 2.5 cm. and weighing 71 Gm. On one surface smooth gray dura was attached in an area 1.5 cm. in diameter at the center. The portions of the tumor adjacent to the dural attachment were smooth and covered by thin transparent tissue. On the other surface of the tumor there was a thin capsule, to which a few fragments of yellow tissue were adherent. As a whole, the external surface was pale yellowish gray. The cut section presented a mottled appearance. Over one surface the thin gray band of dura appeared free from the tumor. Just beneath the dura was a region of firm, white tissue. Tissue of the same character was seen adjacent to other surfaces, and a few islands of similar tissue were observed

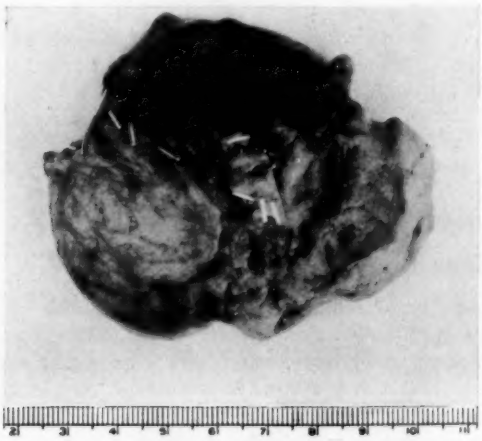

Fig. 1.—Photograph of the specimen removed at operation. Numerous silver clips applied to the dura in order to control bleeding and their absence in the deeper portions of the tumor are shown.

through the tumor. Most of the mass consisted of soft, gray or brown necrotic tissue. One cyst, 15 mm. in diameter, was encountered in the central portion of the specimen. It was lined by smooth, pale gray tissue. Three similar cysts, each approximately 5 mm. in diameter, were noted elsewhere in the tumor.

Microscopic Observations.—Sections were stained with hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid-hematoxylin stain, Mallory's acid fuchsin-orange G-phosphomolybdic acid-aniline blue stain, Verhoeff's stain for elastic tissue, del Rio-Hortega's silver carbonate method for astrocytes, the Cox mercury bichloride modification of the Golgi method and Perdrau's and Laidlaw's methods for connective tissue. Many blocks were chosen from all portions of the tumor to avoid conclusions based on observations on localized groups of cells. In addition, at several levels sections through the entire tumor were cut into blocks, which were arranged in order later to permit a survey of all portions of the tumor at these levels.

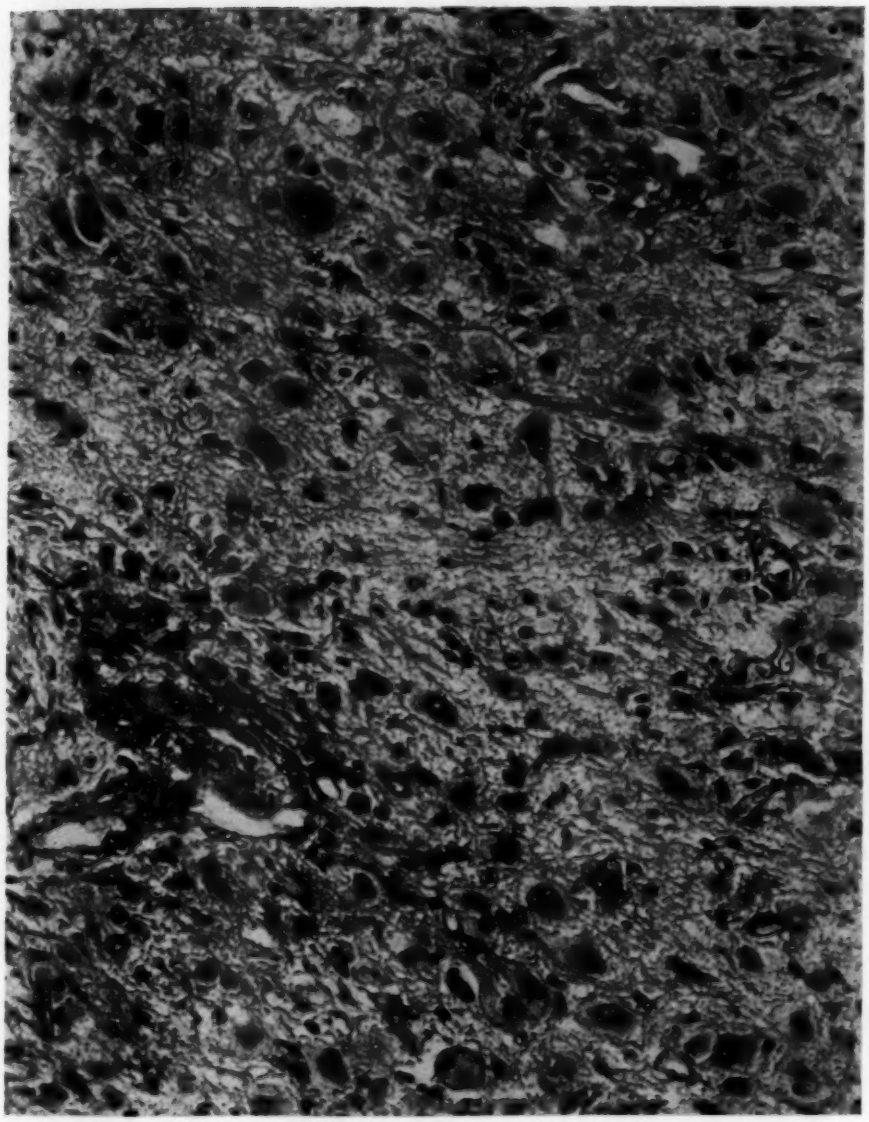

Fig. 2.—Photomicrograph of a section of the tumor, showing the general structure. It is composed of astrocytes among neuroglia fibrils. Phosphotungstic acid hematoxylin stain; × 300.

Over the tumor was a layer of dura, lying free at its edge and attached to the tumor at its center. The dura was composed of layers of collagen arranged mostly in parallel fashion. In the free portion the lower margin was covered by elongate cells with collagen fibers between them. At the point of attachment to the tumor, strands of collagen, usually argyrophilic (reticulum), extended into the tumor. Most of these fibers were associated with capillaries which extended from the dura into the tumor and mingled with its capillaries. Some of the collagen fibers extended from the dura into the superficial portion of the tumor, independent of blood vessels. No remnants of leptomeninx could be identified in the region of adherence of the tumor and dura. In the portions of the meningeal surface in which the dura was not adherent, the flattened cells of the arachnoid formed a layer covering the surface of the tumor.

The tumor itself was composed of glia cells, which were shown by the del Rio-Hortega silver carbonate method to be astrocytes. Cells of protoplasmic and fibrous types were seen. While most of the astrocytes were of the piloid type, plump astrocytes of the type described by Nissl were seen occasionally. In some fields giant astrocytes with two nuclei were observed. These cells presented numerous large, contorted processes and prominent sucker feet. A very small number of astroblasts were encountered. Near the periphery of the tumor the cells were often elongated and contained oval nuclei. However, neuroglial fibers were stained in this region. These cells were shown by the Golgi-Cox method to have fibers arranged close together and parallel to one another attached to the ends of the cells. The shape and arrangement suggested that the form was the result of compression by the expanding tumor. In various parts of the tumor concentrically laminated calcareous deposits (psammomas) were noted.

The cysts were lined by astrocytes which were identical with those of the surrounding tumor. At a short distance from the lumens of the cysts there were dense, irregular groups of collagen fibers. Some of these extended for a short distance among the tumor cells. Except for the portions of the tumor just beneath the point of adherence to the dura and for the walls of the cysts, the collagen was confined to the neighborhood of blood vessels. The tumor contained no elastic tissue except in the laminae of the large blood vessels.

The blood vessels were very numerous. The capillaries formed a dense interlacing, anastomosing network. Some were small and tortuous and others very large. Their walls were composed of a layer of endothelium surrounded by rare collagen fibers. In larger vessels the lumen was frequently narrowed by extensive proliferation of the intima. Hyaline deposits were sometimes seen in the walls of the blood vessels of the tumor. A few vessels evidently represented the leptomeningeal vessels in that location before development of the tumor, because of the width of their mediae of smooth muscle. Some of these vessels were just beneath the surface of the arachnoid, while others were near the connective tissue membrane which separated the tumor from the underlying brain tissue (fig. 7).

There were extensive areas of necrosis throughout the tumor. These patches contained granular, eosinophilic débris with infiltration by polymorphonuclear leukocytes. Compound granular corpuscles were observed in moderate numbers at the periphery of these regions. A few phagocytes contained hemosiderin granules. At the margin of many of these areas there was organization by ingrowth of capillaries in large numbers. Around the capillaries were fibroblastic proliferation and formation of rare collagen fibers.

The inferior margin of the tumor was studied in many sections. It was covered by a layer of connective tissue fibers. In a few sections, fragments of underlying brain tissue were noted, which had adhered to the capsule of the tumor

as it was removed. Here the connective tissue layer was complete and sharply demarcated the tumor from the underlying brain tissue. There were occasional large astrocytes in regular arrangement in the brain tissue. These were separated from one another by the usual elements of the first layer of the cerebral cortex. There was no evidence of connection between the tumor and the brain tissue (fig. 7).

COMMENT

The tumor which has been described was an astrocytoma of the leptomeninges. The cells of the tumor were nearly all well differentiated

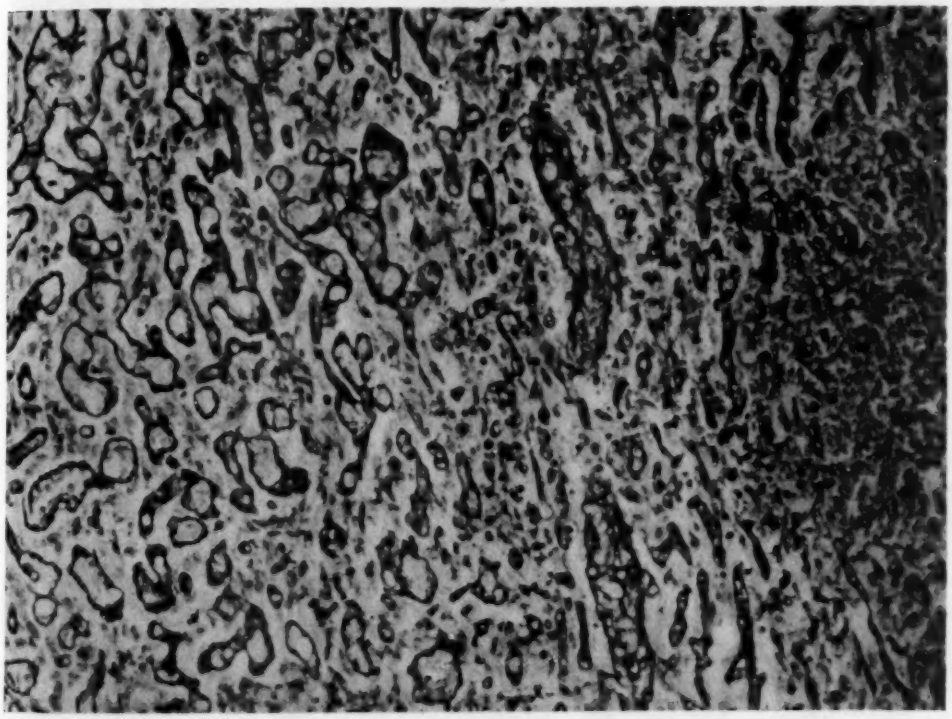

Fig. 3.—Photomicrograph, showing a zone filled with an enormous number of capillaries about an area of necrosis at the right. Most such areas in the tumor are surrounded by areas in which the vessels are more numerous than elsewhere. The capillaries represent organization of necrotic tissue rather than true vessels of the tumor. Laidlaw's method for connective tissue; × 160.

astrocytes of both the fibrous and the protoplasmic type. They differed in no way from those seen in astrocytomas primary in the tissues of the brain or spinal cord proper. There was evidence of rapid growth on the part of these cells in the presence of rather frequent mitotic figures and of cells approaching the astroblast form. The absence of collagen fibers, fibroglia and elastic tissue in intimate association with

the tumor cells also serves to separate this neoplasm from the usual leptomeningeal tumors. There are characteristics, however, that call attention to the fact that this tumor arose in the leptomeninges. Between the brain and the tumor a layer of connective tissue intervened, and no direct connection between the brain tissue and the tumor could be observed. In the few fragments of cerebral tissue which remained

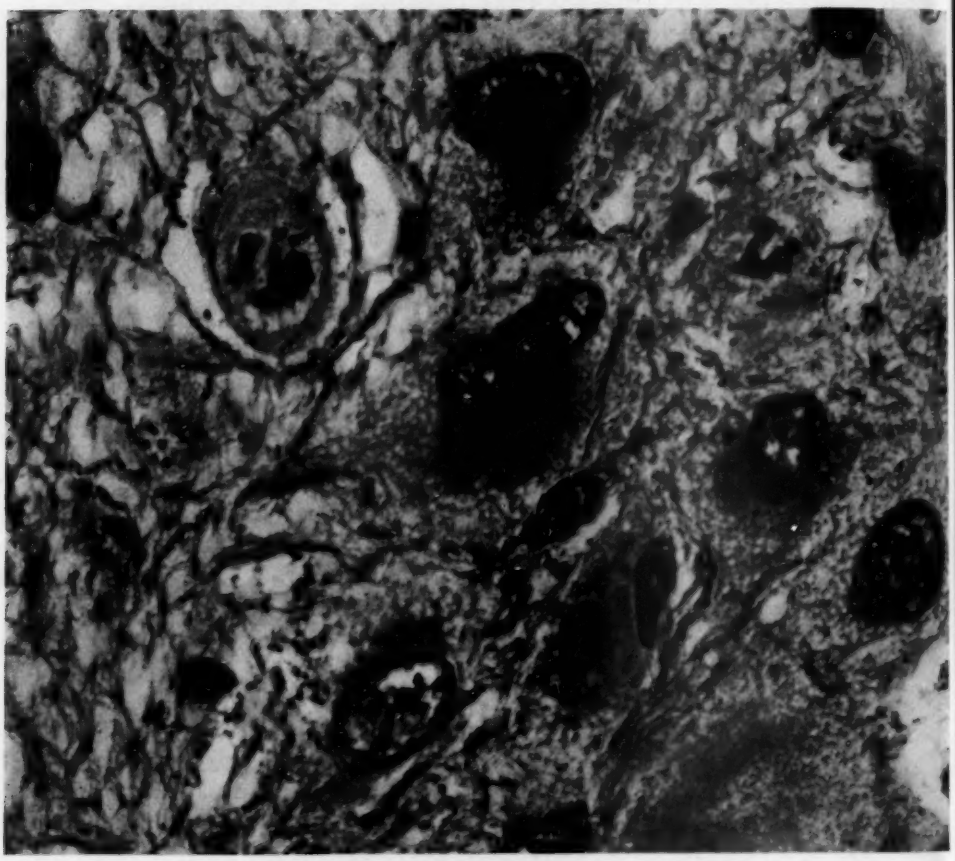

Fig. 4.—Photomicrograph, showing the astrocytes of the tumor surrounded by neuroglial processes. Two mitoses and a binucleate astrocyte are included. Phosphotungstic acid hematoxylin stain; × 1,300.

attached to the capsule of the tumor, the usual structures of the first layer of the cerebral cortex could be identified. There were a few large astrocytes with abundant fibrils in this tissue, familiar as cells noted in areas of reaction to pressure. With del Rio-Hortega's silver carbonate method for astrocytes, these cells stained more readily than the astrocytes of the tumor, recalling that the cells in areas of gliosis

are usually stained more readily by this method than astrocytes found in tumors. The outer surface of the tumor was covered in part with arachnoid tissue and in part with attached dura. Except for the area of

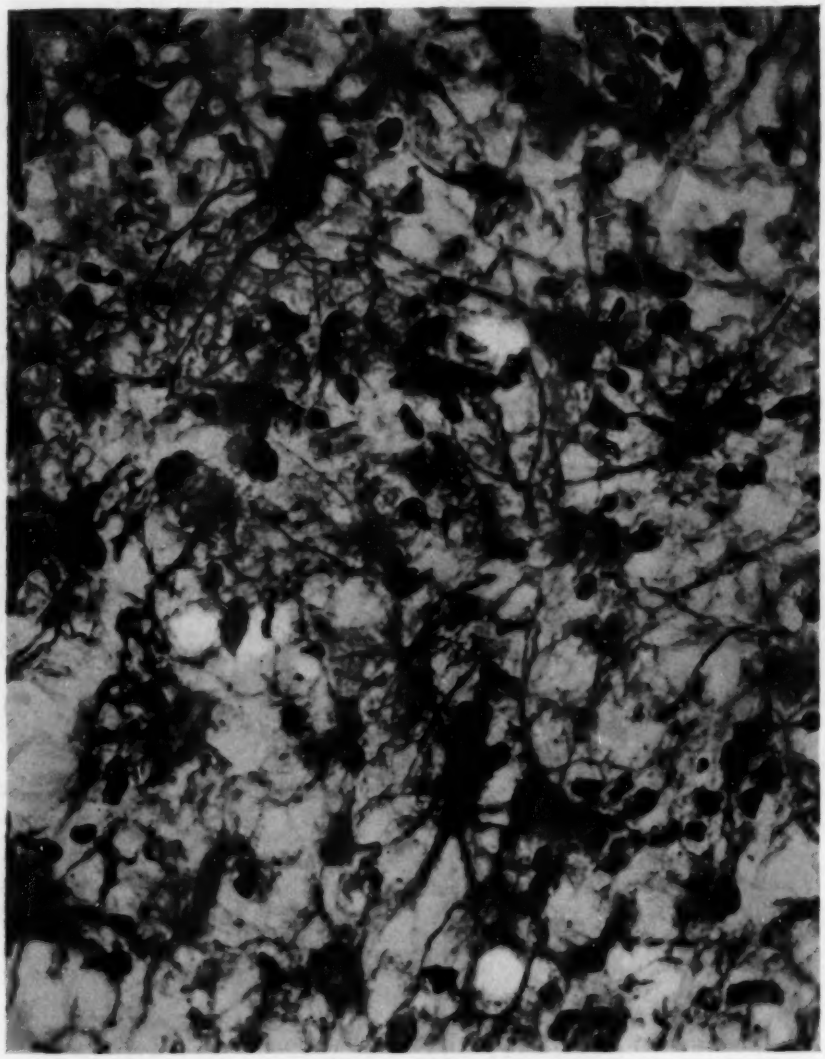

Fig. 5.—Photomicrograph, showing the glial nature of the cells clearly demonstrated by silver impregnation. Some cells are giant astrocytes, while others are smaller and present fewer processes. Del Rio-Hortega's silver carbonate method for astrocytes; × 520.

adherence to the dura, the situation of the tumor was exactly that of the subarachnoid space, since arachnoid cells lay above the tumor and fibrous

tissue of the pia beneath. It has been shown experimentally that a gap in the dura in the absence of injury to the arachnoid is filled with granulation tissue without reaction of the leptomeninges.[6] If the pia-arachnoid is injured, reaction occurs in both the leptomeninges and the dura, with the uniform formation of adhesions between the two membranes.[7] Whether or not these observations indicate a different embryogenesis for the two membranes is irrelevant to the present discussion. The work indicates a method by which any tumor of the leptomeninges may receive a dural attachment as soon as it expands sufficiently to cause necrosis of the arachnoid cells. Since such adhesions contain fibroblasts

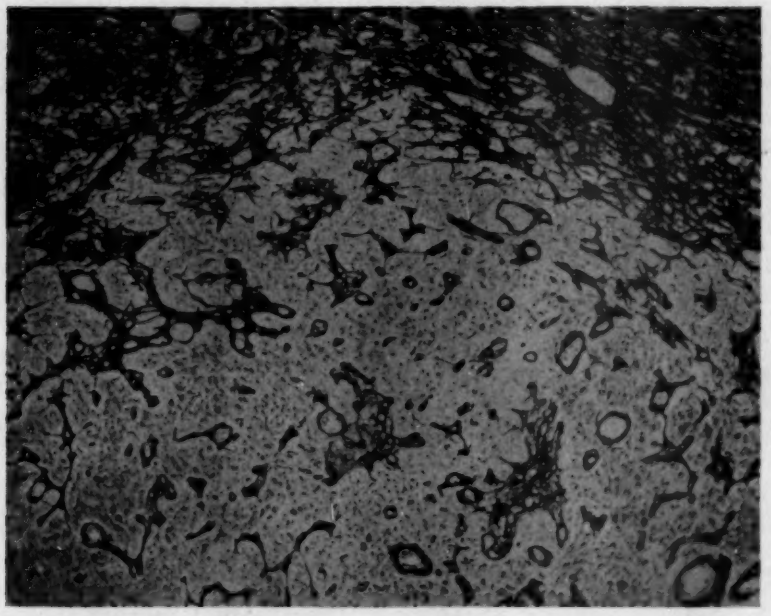

Fig. 6.—Photomicrograph, showing in the upper part of the figure the connective tissue fibers of the dura forming a dense layer pierced by large capillaries. The underlying tumor is richly vascular but possesses no stroma except that about the blood vessels. At the edge of the dura connective tissue fibrils extend for a short distance in the tumor. Perdrau's method; × 70.

and blood vessels, it is to be expected that strands of collagen and capillaries may extend downward for a short distance, at least. That was the situation in the tumor under consideration. Finally, there was strong evidence of the leptomeningeal location of the tumor in its manner of blood supply. It received its entire blood supply from the vessels

6. Sayad, W., and Harvey, S.: Ann. Surg. **77**:129, 1923.
7. Lear, M., and Harvey, S.: Ann. Surg. **80**:536, 1924.

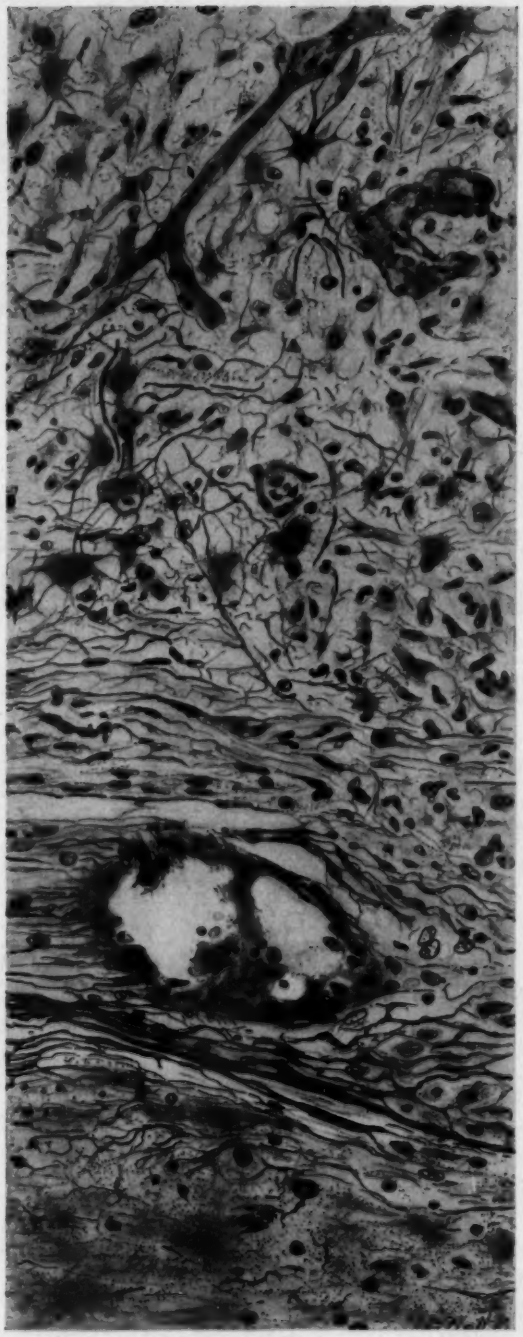

Fig. 7.—Camera lucida drawing (× 280) from a section impregnated by del Rio-Hortega's method for astrocytes. In the upper part of the figure the tumor tissue is shown to be composed of astrocytes, some presenting prominent sucker feet. Just below the center is a large vessel resembling those of the leptomeninges rather than those of the brain. In the lower portion of the drawing is a small amount of underlying brain tissue removed with the tumor. This section shows clearly that the tumor is separated from the brain by a connective tissue membrane.

of the adjacent leptomeninges and dura, which were noted to be enlarged at the time of operation. When the tumor was enucleated, there was no bleeding from the bed of the tumor, and no vessels could be seen entering the tumor from the brain substance.

In spite of the fact that an opportunity was not secured to examine the entire brain, it seems certain that this tumor was primary in its site in the leptomeninges and that it was not a metastasis from an astrocytoma of the brain substance. The clinical manifestations were referred only to the site at which the tumor was seen. When recurrence developed it was at the same site, and no clinical evidence of tumor elsewhere could be observed. When a glioma metastasizes there is usually seeding of the subarachnoid space, and large solitary nodules do not develop.[4] The gliomas giving rise to meningeal gliomatosis are confined to the most rapidly growing of the group, especially medulloblastomas and glioblastomas. The work of Russell and Cairns [8] in regard to metastasis from an astrocytoma will be discussed later.

If it is accepted that a glioma may arise in the leptomeninges, sources of glial tissue in that region must be considered. The arachnoid cells themselves should be mentioned as a possibility. After it was assumed for years that the leptomeninges are of mesodermal origin,[9] evidence, both anatomic [1b] and experimental,[1a] has been advanced to suggest the ectodermal derivation of this structure. Still more recent experiments of Flexner [10] did not confirm the results of Harvey and Burr [1a] and tended to indicate that the important factor in the differentiation of the leptomeninges is not the presence of the neural crest but the formation of the cerebrospinal fluid. This point of view has recently been strengthened by the results of other workers.[11] Even if the neuro-ectodermal origin of the leptomeninges is correct, it would be unusual for cells to undergo so remarkable a process of dedifferentiation and redifferentiation in the formation of a tumor without leaving traces of the progression. There are no cells resembling arachnoid cells among the astrocytes of the tumor, nor are there any cells which could be interpreted as indicating stages in the transformation.

Glia cells have been demonstrated in cranial nerves for some distance from their point of exit from the brain. However, no cranial nerves leave the brain near the site of the tumor described here. The occurrence of glia in association with the minute nerves supplying the leptomeninges themselves has not been demonstrated.

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8. Russell, D., and Cairns, H.: J. Path. & Bact. **33**:383, 1930.

9. Weed, L.: The Meninges, with Special Reference to the Cell Coverings of the Leptomeninges, in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 611.

10. Flexner, L.: Contrib. Embryol. (no. 110) **20**:31, 1929.

11. Weed, L.: Brain **58**:383, 1935.

There remains as a possible source of glia in the leptomeninges the neuroglia nests first described by Wolbach.[12] Since that time occasional reports[13] have confirmed his observations. Though there is little to add to the published descriptions, the close relationship between the neuroglia nests and the tumor under consideration warrants a review of their detailed structure. Wolbach's original case occurred in a patient 10 months old, who presented spina bifida with hydrocephalus and congenital rhabdomyoma of the heart. The dorsal surface of the cervical enlargement of the spinal cord was covered with so many small nests of neuroglial tissue that the leptomeninges had a sanded appearance. The number gradually decreased caudad. Scattered nests of neuroglia were observed in the posterior nerve roots, on the superior surface of the posterior portion of the velum interpositum and within the parietal part of the cortex. Two of these inclusions contained ependyma-lined canals. In three cases studied in this laboratory, the general distribution of the nests corresponded closely to those in Wolbach's original case. One instance occurred in a previously normal child of 7 years who died of acute anterior poliomyelitis; a second, in an infant 8 weeks of age who died of chronic bronchopneumonia and fibrosis of the pancreas without clinical evidence of abnormality of the central nervous system, and a third, in a child of 8 years with hydrocephalus. It is to be emphasized that in two of the cases the neuroglia nests were not associated with clinical or pathologic evidence of other congenital abnormalities of the central nervous system.

The neuroglia nests varied in size from 0.1 to 1.5 mm. in the greatest diameter. They were situated between the pia and the arachnoid. In all instances a layer of connective tissue intervened between the two, the intact pia. The nests were surmounted by a layer of connective tissue fibers and arachnoid cells. Thus, they lay wholly within the subarachnoid space.

Most of the nests were oval. The smallest were round, while the largest spread out in sheets, following the contour of the leptomeninges. Neither the meninges nor the underlying tissue was much distorted by the nests. Some of the larger nests presented irregular finger-like projections, while others were composed of two masses of neuroglia connected by a band of neuroglia fibers containing few cell bodies, which arched over a blood vessel. The nests were situated usually, but not always, in proximity to medium-sized blood vessels, approaching their adventitiae without extending into their walls. Occasional nests along the spinal cord were observed near nerve roots.

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12. Wolbach, S.: J. M. Research **16**:495, 1907.

13. Kernohan, J.; Woltman, H., and Adson, A.: Arch. Neurol. & Psychiat. **25**:679, 1931.

Each neuroglia nest was surrounded by a delicate investment of connective tissue, derived from both the pia and the arachnoid. From this investment a few reticular fibers extended into the nests. The nests contained small blood vessels derived from the vessels of the subarachnoid space. Otherwise, they were composed entirely of glia

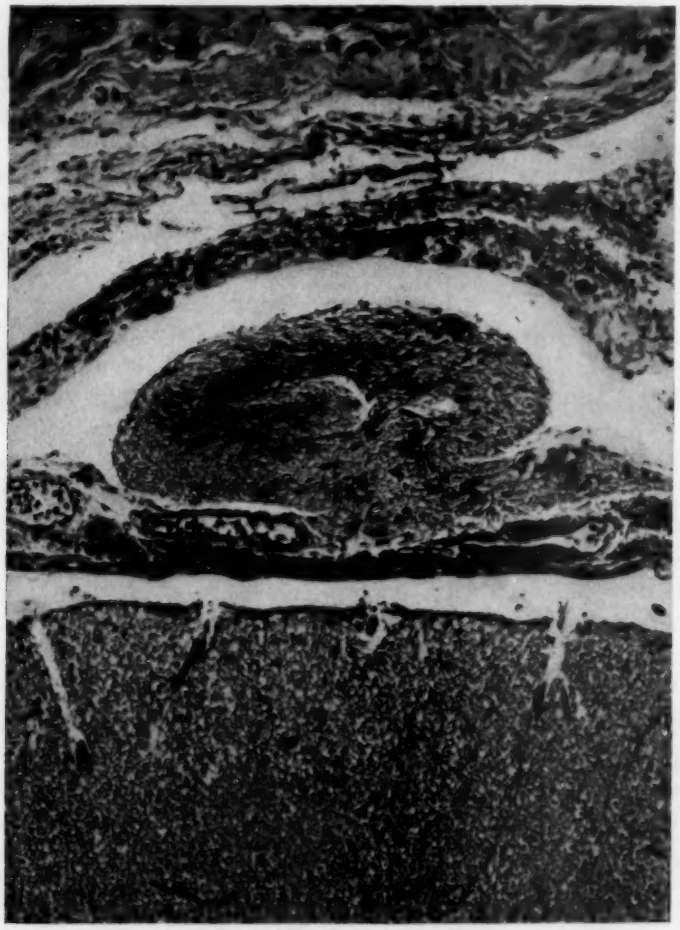

Fig. 8.—Photomicrograph of a neuroglia nest in the subarachnoid space. In the upper part of the figure are shown the connective tissue fibers of the dura; in the lower part the spinal cord. The neuroglia nest lies between the pia and the arachnoid. The central clumping of cell bodies and the peripheral orientation of fibers are apparent. There is no connection between the neuroglia nest and the tissue of the spinal cord. Phosphotungstic acid-hematoxylin stain; × 300.

cells and fibers. There were no nerve cells in any of the nests studied. The nuclei were grouped at the center of the nests, while the peripheral

portion was composed of a dense arching network of neuroglia fibrils. The fibrils of the central portions, fewer than at the periphery, were usually irregular in arrangement but occasionally formed small bands of parallel fibers. The cells were adult astrocytes with nuclei and cytoplasm similar to those of the astrocytes within the underlying brain or spinal cord. Mitoses were not encountered. Occasionally smaller nuclei were observed among the nuclei of the astrocytes about which no processes were demonstrated by the phosphotungstic acid-hematoxylin stain. These nuclei resembled those of oligodendroglia cells. Unfortunately, no material was fixed properly for the silver impregnation of these cells.

A few instances of neuroglia nests or similar structures have been described which depart somewhat from this type. In the case reported by Buckley and Deery,[14] there were multiple neuroglia nests over the frontal portion of the cortex. These nests differed from those just described in that a connection with the cerebral cortex could be demonstrated, but they resembled them in that they were composed of pure neuroglia without nerve cells or fibers. Freeman [15] described a case of cortical heterotopia in the subarachnoid space. This tissue presented a cysto-architecture resembling that of the hippocampal gyrus. It differed from neuroglia nests in the presence of nerve cells and fibers as well as in arrangement. Extension of neuroglia along occluded blood vessels and the migration of glial elements into the subarachnoid space in cases of porencephalus [16] seem to be the result of inflammation or the consequence of a degenerative process and not to be related to neuroglia nests. Glial heterotopias may also occur in the brain substance or beneath the ependyma.[17]

As an explanation of the neuroglia nests it has been suggested that they are undifferentiated neoplastic tissue which becomes differentiated to form heterotopias.[18] The regularity of the arrangement of their cells and fibers, as conspicuous in the new-born as in older persons, the absence of mitoses and the discrete character of the nests speak strongly against such an interpretation. The alternative explanation that the nests represent invasion of the leptomeninges in embryonic life seems far more consistent with the general appearance of the nests, their presence at birth and their occurrence in patients with other anomalies of the central nervous system. It should be repeated, however, that neuroglia nests can occur in the absence of any other malformations

14. Buckley, R., and Deery, E.: Am. J. Path. **5**:459, 1929.
15. Freeman, W.: Arch. Path. **2**:352, 1926.
16. Globus, J.: Arch. Neurol. & Psychiat. **6**:652, 1921.
17. Brunschweiler, H.: Rev. neurol. **1**:1019, 1930. Kernohan, J.: Arch. Neurol. & Psychiat. **23**:460, 1930. Wolbach.[12]
18. Bailey, P., and Bucy, P.: Am. J. Cancer **15**:15, 1931.

of the brain or spinal cord. It has been pointed out that in certain patients with increased intracranial pressure, multiple herniations of brain tissue through the pia result.[19] In this location the neuroglia cells survive and proliferate while the neurons disappear. The herniations differ from the neuroglia nests in their connection with the brain or spinal cord and in their lack of the architectural arrangement so constant in neuroglia nests. Neuroglia nests occur at times in patients who have never given evidence of increased intracranial pressure. The multiple herniations are seen in the cerebrum and cerebellum, while the neuroglial nests are usually most numerous along the spinal cord.

Neuroglia nests of the type discussed in detail offer a source for the tumor described earlier in this paper and for true glial tumors of the meninges in general. Both the tumor and the nests occupied the subarachnoid space, with a membrane of connective tissue between them and the underlying nerve tissue and with arachnoid (or its remaining fragments in the case of the tumor) over them. The nests contained adult glia cells and thus furnish a source of glia without the assumption of an elaborate process of dedifferentiation and redifferentiation. While direct proof is lacking, this seems by far the most likely explanation of their histogenesis.

In a review of the literature on neuroglia nests and tumors which might take origin from them, the work of Russell and Cairns [8] in regard to metastases from an astrocytoma deserves more than passing mention. These authors described an astrocytoma of the right optic thalamus which in its growth had free access to the subarachnoid space. At the posterior edge of the tumor, dorsal to the midbrain, there were numerous small islands of astrocytes, each island being invested with connective tissue and well vascularized. These islands were located in the leptomeninges between the pial fibers and the arachnoid cells. In the leptomeninges covering the spinal cord similar nodules of astrocytes were observed, most frequently on the dorsal surface of the cord, but a few were seen on the anterior aspect. The nodules resembled neuroglia nests in size. Microscopic examination showed that the nodules were composed of astrocytes without giant cells or mitotic figures. The cell bodies were mostly at the center of the nodules, while the periphery was composed chiefly of fibers oriented at right angles to the surrounding connective tissue fibers. The nodules, then, presented the architectural arrangement of cells and fibers so often encountered in neuroglia nests. The case has been interpreted as one of cerebral astrocytoma with multiple metastases. It seems possible also to interpret it as an instance of multiple neuroglia nests with a glioma arising in one of them. The

19. Wolbach, S.: J. M. Research **14**:153, 1908.

reasons for this interpretation are the histologic identity of the nodules and neuroglia nests, especially in respect to the central location of the cell bodies and the peripheral orientation of the fibers, the location of the nodules in the subarachnoid space, the presence of the nodules at the edge of the tumor, the histologic character of the tumor (astrocytoma) and the absence of similar observations in other cases of this type of tumor. At autopsy the tumor was so large that its original point of origin is a matter of conjecture.

In a paper dealing with intramedullary tumors of the spinal cord, Kernohan, Woltman and Adson [13] referred to three extramedullary tumors of this region which belonged to the glioma group and suggested their derivation from neuroglia nests. The tumors were removed from the subarachnoid space and were attached to the pia. Their origin could not be traced to the substance of the spinal cord. Further details were not given.

Thompson,[20] in describing a series of three "leptomeningiomas" of the spinal cord, included two with the usual histologic structure of meningioma. In his first case, however, the characteristics of the tumors to which this paper is devoted were presented. The tumor was exposed at operation between the seventh and the eighth spinal segment and was easily removed. There was no recurrence at the end of seven years. Histologically, the tumor was made up of very large cells, which were long and tapering or triangular. These cells were associated with an abundant feltwork of fibers, which stained deep blue with the phosphotungstic acid-hematoxylin stain and red with Masson's method. The cells resembled glia cells, and the fibers, neuroglia processes. The tumor contained a large artery with a thick muscular layer and a wide adventitia. The enclosed vessel resembled arteries of the leptomeninges and not those of the substance of the spinal cord. This observation is similar to that in the case reported here.

A meningeal tumor described by Hillel [21] was located in the middle fossa. It contained many astrocytes in some areas, and there were extensive necroses. There was much intracellular pigment, which did not stain like iron. The varied histologic structure of the tumor and the character of the pigment raise the question of malignant melanoma of the leptomeninges with inclusion of cerebral tissue and reactive gliosis. The growth of astrocytes seems too extensive and rapid for the validity of this interpretation. Yet one would be hesitant in including this tumor in the group of true gliomas of the leptomeninges.

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20. Thompson, T.: Lancet **1**:325, 1929.
21. Hillel, M.: Bull. Assoc. franç. p. l'étude du cancer **17**:229, 1928.

The tumor reported by Roussy, Cornil and Leroux [22] as meningeal tumor of the glial type was a meningioma and does not belong in this group.

On the basis of the study of the present material and of the cases collected from the literature, it seems that there exists a small group of tumors of the leptomeninges which are situated in the subarachnoid space and present the histologic characteristics of gliomas. The presence of neuroglia nests in these locations offers a ready explanation of their histogenesis. The present case and the cases reported in the literature were instances of astrocytoma. In addition, it would be possible for ependymoma to occur in the leptomeninges because of the occasional presence of ependymal canals in the nests of neuroglia tissue. These neoplasms represent a specific type of tumor of the leptomeninges with a histogenesis peculiar to it. The cases are too few to offer valid data in regard to prognosis. Yet it may be that their clinical manifestations are to be judged more on the basis of the behavior of the similar glioma in the brain substance than on the life history of the meningioma, due regard being given, none the less, to their accessible location and their limiting connective tissue membrane.

SUMMARY

Clinical and pathologic studies are presented in the case of a patient with a tumor of the left frontal portion of the leptomeninges having the histologic characteristics of astrocytoma.

Of the various possible sources of glia in the leptomeninges, neuroglia nests offer the most probable explanation of the histogenesis of this tumor. These are anomalous inclusions of neuroglia in the subarachnoid space. They may occur in instances of extensive congenital malformations of the central nervous system, and they have also been encountered without association with other anomalies of the brain or spinal cord.

In a review of the literature, a small group of leptomeningeal tumors has been collected, which presents the histologic characteristics and anatomic situation of the tumor described in this paper. True gliomas of the leptomeninges, therefore, make up a small but distinct group of meningeal tumors.

22. Roussy, G.; Cornil, L., and Leroux, R.: Rev. neurol. **39**:294, 1923.

CEREBRAL NEURO-EPITHELIOMA

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Neuro-epithelioma of the cerebrum is rare; Cushing [1] reported but 1 instance in 2,023 verified cases of intracranial tumor; Bucy and Muncie [2] seven years ago found reports of only 5 bona fide cases in the literature and added 1, and Tello and Herrera [3] reported the seventh case in 1934. The tumor, arising possibly from the primitive spongioblast, is, however, not an uncommon type of neoplasm of the spinal cord [4] and also occurs frequently in the retina. Its histologic derivation is still subject to involved controversy.

REPORT OF A CASE

History.—A. G., a French-Canadian logger aged 65, married, was seen at the outpatient department of the University of Oregon Medical School on Oct. 5, 1933, complaining of blurred vision in the left eye, headache and dizziness. His father had died at 62 of apoplexy and his mother at 74 of paralysis; sixteen siblings were well; his wife was 61, and he had five children, all of whom were in excellent health. He had had mumps, measles and pneumonia; his leg had been broken in 1900, and a "growth" had been removed from the left breast and axilla several years previously. A year before he was first seen at the outpatient clinic he had had a sudden spell of dizziness, lasting thirty minutes, while sitting quietly in a barber's chair having his hair cut; the episodes of dizziness thereafter recurred once or twice a week, and for the week prior to his visit they had been present daily, especially on exertion; he had also had severe daily headaches for a month, each lasting from four to five hours, which preceded the individual attacks of dizziness. He had noted blurred vision in the left eye for a similar length of time. He had never lost consciousness during any part of an attack.

Examination.—Examination showed dental caries, pupils 2 mm. in diameter, mild arcus senilis and good vision in the right eye but practically complete loss of vision in the left. In the Weber test hearing was referred to the left ear. There was potential inguinal hernia on the right side. His weight was 170 pounds (77.1 Kg.), and his height, 70 inches (177.8 cm.). The pulse rate was 68 per

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From the neuropathologic section of the Department of Pathology, University of Oregon Medical School.

1. Cushing, H.: Intracranial Tumors: Notes upon a Series of Two Thousand Verified Cases, with Surgical-Mortality Pecentages Pertaining Thereto, Springfield, Ill., Charles C. Thomas, Publisher, 1932.
2. Bucy, P., and Muncie, W.: Am. J. Path. **5**:157, 1929.
3. Tello, J. F., and Herrera, J. M.: Arch. de neurobiol. **13**:61, 1934.
4. Fraenkel, A., and Benda, C.: Deutsche med. Wchnschr. **24**:442, 1898.

minute, and the blood pressure, 138 systolic and 90 diastolic. The Wassermann reaction was negative. The hemoglobin concentration was 90 per cent; the leukocyte and differential counts were normal, and the sedimentation rate was 5 mm. in fifteen minutes and 33 mm. in forty-five minutes. An otologist seen in consultation reported that the dizziness was not of labyrinthine origin. Though refraction was performed, the optic fundi were not examined.

Course.—The patient entered the Multnomah Hospital on Sept. 22, 1934, with a history of having been in bed for the preceding three months because of weakness; he had often had a sensation of "knotting in his stomach," but emesis had occurred only rarely. At 3 a. m. on the day of entrance to the hospital he became delirious and was unable to recognize attendant relatives; a few hours later he subsided into coma, with respiration of the Cheyne-Stokes type. The pupils reacted sluggishly to light. Though the tendon reflexes were brisk, the superficial reflexes were absent, and there was poor response to painful stimuli on the right half of the body. He remained in semistupor and swallowed with difficulty. There was incontinence of feces and urine; the temperature ranged from 99 to 100.2 F., and the pulse rate varied from 88 to 110 and the respiratory rate from 18 to 32 per minute. The urea nitrogen content of the blood was 61.5 mg. per hundred cubic centimeters of blood. Though the blood pressure was 114 systolic and 88 diastolic and the radial arteries were found to be only moderately thickened, the tentative diagnosis made was disease of the coronary vessels and probable cerebrovascular accident. On Sept. 26, 1934, the patient became cyanotic, and there were numerous râles in the chest. He died at 7:45 p. m. Necropsy on the embalmed body was performed seventeen hours after death.

Anatomic Diagnosis.—The diagnosis was multiple hemorrhages of the brain, hypostatic edema and hemorrhages of the lungs, moderate dilatation of the right side of the heart, generalized chronic passive hyperemia, moderate emaciation, bilateral chronic arteriosclerotic nephritis, atrophy of the testicles, congenitally undescended cecum, mild hypertrophic rachitis and obliterative fibrous pleuritis. The cerebral neoplasm was discovered in the routine examination of the formaldehyde-fixed brain several months subsequently.

Macroscopic Examination of the Brain.—The brain was evidently that of an elderly person. There was moderately advanced arteriosclerosis of the major vessels of the base, and all the empty arterial lumens remained round and patent, even though no gross arteriosclerotic plaques were visible in their walls. The arachnoid was everywhere thickened and gray over the convexity and base, possibly a little more markedly in the posterior portion of the right parietal region. The cortical gyri showed moderately advanced diffuse atrophy, and the sulci varied from 1.5 to 5 or 6 mm. in width, the widening being mostly confined to the frontal and parietal lobes of the right hemisphere. Spreading upward in the left sylvian fissure and to a less degree in the right was a hemorrhagic extravasation. This had distended the inferior operculum and overlay the entire surface of the left island of Reil; an estimate of the total amount would not exceed from 18 to 20 cc. The hemorrhage was confined solely to the subarachnoid spaces externally and had its origin in the anterior half of the tumor to be described later, but when coronal sections were made of the brain, it was seen that the anterior perforated substance on the left, beneath the anterior commissure, was infiltrated and disrupted by a compact mass of extravasated blood; this also extended mediad as a sheath about the left half of the anterior commissure. No lacunar degeneration was evident. The lumens of the arterioles of the left striatum were distended with blood, but this was not present on the right.

A globular, pinkish-gray, somewhat warty tumor, about 32 by 30 by 28 mm., occupied the entire interpeduncular space anterior to the pons (fig. 1) and extended forward to the olfactory trigon bilaterally, bellying the optic nerves upward (especially the left) and incasing the left carotid artery for probably 12 mm. or more. The growth had intruded into the subarachnoid spaces on the left and created an asymmetrical warty accessory nodule, 12 by 12 by 15 mm. (included in the original dimensions of the entire tumor), which extended into the sella and posteriorly as a lappet into the extreme anterior part of the left cerebellopontile angle. The anterior half of the tumor was infiltrated with blood, but this did not extend into the posterior third of the tumor. The membranous floor of the third ventricle was apparently intact in the right half of the brain, and this was also true of the floor of the ventricle, where a paper-thin lamella of glial tissue could be seen to pass entirely underneath the nodule, which must, therefore, have only protruded into the third ventricle at this point (fig. 1). The infundibular stalk was distended by the tumor to a diameter of 4.5 mm. The superior surface of the growth presenting toward the lumen of the third ventricle was also overlaid by a

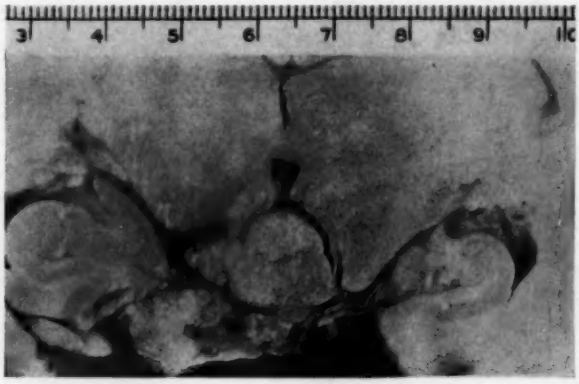

Fig. 1.—Photograph showing the texture and relations of the tumor. The apparent intraventricular nodule is actually enveloped by a complete lamella of glial tissue and hence is an invagination from without.

delicate glial membrane in unharmed portions of the tumor. However, on making further close-set coronal sections immediately anterior to the hypophyseal stalk, one observed that the tumor in this region infiltrated the lower 9 mm. of the right lateral wall of the third ventricle to a depth of from 3 to 4 mm. and that just medial and inferior to this the growth had pierced the floor to reach the leptomeninges. A cleft in the tumor existed at this level, which clearly defined the mural infiltration from the major mass projecting into the lumen of the ventricle from without. The tumor therefore apparently took its origin at this location and, pushing toward the left both within the ventricle to a mild degree and without (to a marked extent), came to fill the entire interpedunculomamillary region.

Microscopic Examination.—The tumor was compact and highly cellular and showed little degeneration in the sections examined; architecturally, it tended in the peripheral portion (and to a less degree in the interior) to form tubules and strands (fig. 2*A*), but throughout its entire structure abundant true rosettes were

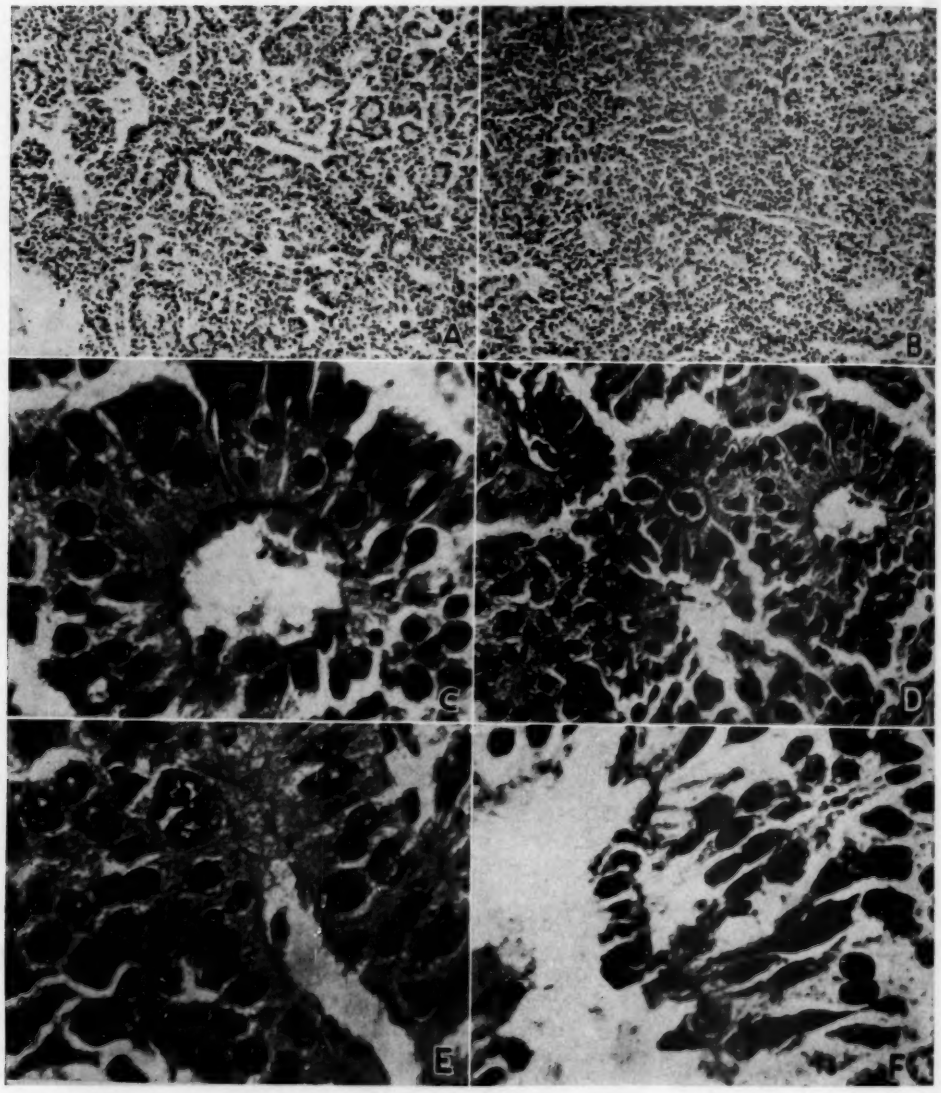

Fig. 2.—Photomicrographs of the tumor showing: (*A*) the arrangement of foldings and tubular and cordlike formations (hematoxylin and eosin, × 40); (*B*) the great abundance of true rosettes (hematoxylin and eosin, × 40); (*C*) rosettes, presenting blepharoplasts and cilia (iron hematoxylin); × 390); (*D*) rosettes and blackened material in the lumens (iron hematoxylin; × 200); (*E*) the occasional tubular arrangement and manner in which the acute ends of the cells constitute a polygonal pavement for the lumen (iron hematoxylin; × 390), and (*F*) chromatin extruded into the processes beyond the limiting cuticular membrane (iron hematoxylin; × 390).

present in practically every field. In sections overstained with iron hematoxylin (Heidenhain technic for blepharoplasts), the cytoplasm of the cells was fairly distinctly seen (figs. *2 E* and *F* and *3 A* to *D*) as being of elongated columnar or irregular polygonal outline. The base of the columnar cells abutted solidly against traversing vessels but showed equal orientative catholicity for traversing connective tissue or even for the outer boundaries of the tumor itself. Although most of the tumor substance tended to cordlike and pseudo-acinar arrangement (without anywhere a suggestion of an external neuro-embryonic limiting membrane), there were occasional broad areas of sheetlike, flowing polygonal cells (figs. *2 E* and *3 C*) interspersed through the more typical architecture.

Fully two thirds of the rosettes showed excellent blepharoplasts, ranged just beneath a delicate internal limiting membrane (figs. *2 C* and *D*, and *3 A*); the blepharoplasts were usually in the form of a diplosome, but occasionally four such granules were noted immediately beneath the membrane; an extruded nucleolus could very occasionally be observed near a nucleus, but no other cytoplasmic inclusions were demonstrable. Shaggy indubitable cilia were seen stemming into the lumen from groups of cells in a few rosettes; most lumens, however, bore only confused agglutinated granular material (fig. *3 A*), extending as a nondescript mass into the lumen of the rosette; here and there (figs. *2 D* and *3 B*) the material beyond the internal limiting membrane had undergone a hyalinoid change and lay free as a blackened aggregation within the lumen. The acute ends of the columnar cells abutted together as a pavement to form the lumen (fig. *2 E*) of the rosettes and canaliculi, and at times nuclei projected beyond the membrane, or, rarely, chromatin material was extruded entirely beyond it (fig. *2 F*).

True neoplastic cytologic characteristics were evident diffusely but were especially marked in regions not containing rosettes; multinucleated cells, giant cells and polychromatism could be readily demonstrated (fig. *3 C*).

Specific metallic staining was singularly unilluminating. The Perdrau stain showed a moderately abundant reticular framework (fig. *3 E*) tending to incase acini, but it was often far more sparse than that shown in the illustration. Not a single element of this tumor could be impregnated with gold sublimate, though it should be noted that Tello [8] was able to impregnate a few astrocytes in the cerebral neuro-epithelioma he described. The phosphotungstic acid-hematoxylin stain showed not a single glial fiber, and no spongioblasts could be specifically impregnated, although Tello was able to demonstrate many of these in the tumor he reported. Several methods were tried for demonstration of early neural elements, but only by the use of Cajal's alcohol-silver-hydroquinone method, with our material which had undergone long fixation with formaldehyde, was it possible to demonstrate sharply the dense tails (fig. *3 F*) of a few unipolar neuroblasts. The Nissl stain was of no specific value.

HISTOLOGIC CLASSIFICATION

Although the histologic picture just described definitely establishes this neoplasm as a neuro-epithelioma, according to accepted definitions, it leaves as open as before the question of the nature of the cells composing it, their embryologic derivation and the systematic place of the tumor in the schema of neoplasms arising from tissues derived from the primitive neural tube.

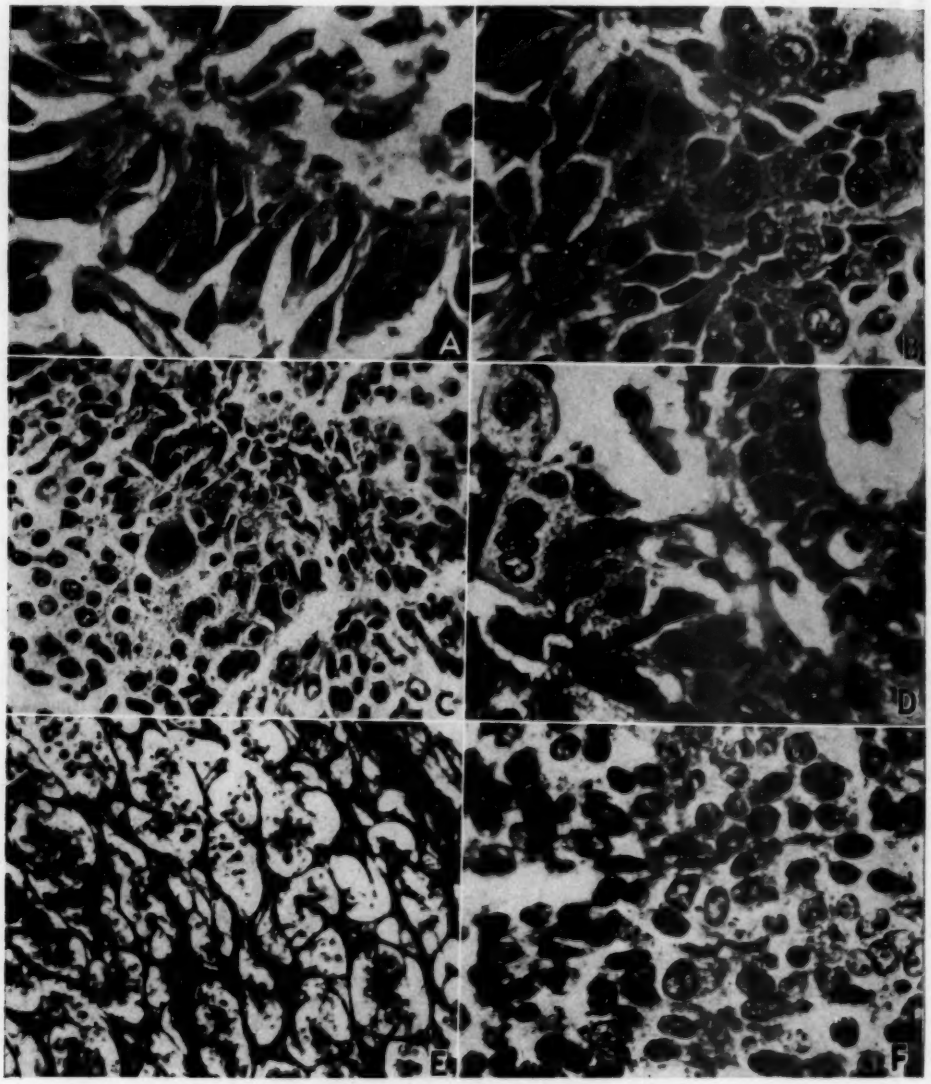

Fig. 3.—Photomicrographs showing (*A*) the unitary structure of the cells composing the rosettes (iron hematoxylin; × 390); (*B*) not only nuclear types but suggestions of the sheetlike cellular material between the rosettes (iron hematoxylin; × 390); (*C*) neoplastic polychromatism, multinucleation and gigantic cells (× 200); (*D*) the cellular formation reminiscent of medullary epithelium on the right (× 390); (*E*) abundant reticular framework in many parts of the tumor (Perdrau stain; × 100), and (*F*) specific staining of the polar processes of two neuroblasts, showing the characteristic arrow-head termination of the upper process (about × 350).

Since the tumor is common in the eye (where it was first described [5]) and the optic vesicle is a direct evagination of the embryonic neural tube, hope of ultimate satisfactory classification of this obscure cerebral neoplasm necessitates a circuitous approach, weaving between the neural and the ophthalmic pathologic picture.

THE ROD AND CONE ANALOGY

Since it is hypothesized that the retinal tumor homologizes with the rod and cone cells, it will be well to examine the structure and derivation of these cells.

Retinal Cytologic Embryology.—The distal pole of the optic vesicle, budding from the neural tube, invaginates to form the optic cup; consequently, the initial lumen between the two layers thus formed is analogous to the cerebral ventricle, which is lined by ependyma; the outermost layer of the new-formed cup regresses to form ultimately only the pigmentary layer of the retina, while the inner layer of the cup becomes transformed into retina proper, the rods and cones themselves thereby homologizing as elaborations of ependymal cilia (as described later in the paper). Of the nerve structures in the retina proper, the multipolar ganglion cells are first to develop, the axon being laid down to the papilla and all the dendrites atrophying except those ascending to the bipolar cells distally. The rod and cone cells, "peculiar structures that can be classified neither as nerve- nor neuro-glial cells,"[6] migrate to the ocular external limiting membrane (the neuro-embryonic internal limiting membrane), sending a process thence backward into the external reticular layer and later developing the rods and cones themselves from the opposite pole of the cell. The latter structures then project beyond the external limiting membrane and are embedded among the cells of the pigment layer. The internal granular layer containing the bipolar neural cells is intercalated between these two layers (the ganglion and the external granular layer), with dendrites ramifying in the external reticular layer and axon synapsing in the internal reticular layer with the dendrites of the multipolar ganglion cells. The internal granular layer, however, is not homogeneous; not only do there lie in it neural bipolar cells, but in its inner portion are amacrine cells of disputed function (spongioblastic, i. e., glial, according to Müller and Kallius, and neural, according to Cajal) and the nuclei of the fibers of Müller. The fibers of Müller are glial and homologize with the most primitive ependymal cells of the neural tube, stretching from the internal limiting membrane of the ventricular cavity to the external limiting membrane on the outer surface of the neural tube. Other differentiated glial structures present in the retina are: astrocytes, which are observed in the nerve fiber (ninth) and internal plexiform (seventh) layer,[7] and oligodendroglia, which have recently been shown to be present in the ganglionic (eighth) layer.[8]

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5. Flexner, S.: Bull. Johns Hopkins Hosp. **2**:115, 1891.

6. Keibel, F., in Keibel, F., and Mall, F. P.: Manual of Human Embryology, Philadelphia, J. B. Lippincott Company, 1912, vol. 2, p. 243.

7. Ramón y Cajal, S.: Histologie du système nerveux de l'homme et des vertébrés, Paris, A. Maloine, 1911, vol. 2, pp. 299 and 316. Greeff, C. R.: Arch. f. Augenh. **29**:324, 1894.

8. Marchesani, O.: Arch. f. Ophth. **117**:575, 1926. Enriquez, L.: Bol. Soc. españ. de hist. nat. **26**:1, 1926.

Retinal Nuclear Characteristics.—In the internal granular layer of the retina the bipolar cells, with their distinctive vesicular nuclei (6.5 by 7 microns) and the single nucleolus suspended by a fine linin network, are practically unmistakable and are readily identified as of neuronal character; they compose about 65 per cent of the cells. The nuclei of the cells of Müller (possibly from 5 to 12 per cent of the cells) and their cytoplasmic characteristics identify them equally indubitably as glial. In the internal portion of this layer, however, are the abundant amacrine cells (possibly from 20 to 35 per cent of the cells); these are the spongioblasts of older authors, and the layer was even, by cautious inadvertence, denominated thus occasionally by Cajal as *la couche des spongioblastes.* These cells were demonstrated by Dogiel to be without axons (confirmed by Cajal), to stain as neurons (intravitam staining with methylene blue, Dogiel, and staining of cytoplasmic neurofibrils, Cajal) and to "synapse" principally in the inner plexiform layer. A few aberrant amacrine cells may be observed occasionally displaced centrally into the ganglionic layer. The nuclei of amacrine cells often lie horizontally (as do the cells), are usually slightly smaller than the nuclei of bipolar cells and have a relatively dense nuclear membrane; their nuclear chromatin is less dispersed and is often aggregated (in the guinea-pig) into from two to four clumps suggesting nucleoli; solitary well formed nucleoli are practically never seen in these cells; such nuclear characteristics are as readily recognized in the displaced amacrine cells (seventh and eighth layers) as in the cells of the internal granular layer itself; these cells are more frequent toward the macula, where they often aggregate into groups of from two to four cells. In the human material available to us the nuclear characteristics of the amacrine cells were observed to be practically identical with those mentioned in the preceding description, except that the nuclei were numerically more profuse and usually showed from four to five nucleolus-like condensations of chromatin.

In the external granular layer (the nuclei of the rod and cone cells) the nuclei are observed to be smaller (from 5 to 6 microns), with dense agglutinated mossy chromatin and an exceedingly delicate nuclear membrane; the chromatin is not infrequently aggregated into two or three fairly definite transverse bands. Near the macula from 30 to 50 per cent of the cells have nuclei of vesiculiform composition and a single plump, mossy nucleolar aggregation, although the size of the nucleus remains the same. Rarely, the nuclei of this layer are observed lying entirely beyond the external limiting membrane, and there also may occur occasional extrusions of solid bleblike chromatin from the nucleus into the base of the rods and cones themselves, beyond the external limiting membrane. The layer can be observed to contain no other types of nuclei.

Ocular Glioma.—According to Parsons,[9] glioma of the retina (of unspecified types) arises from the various layers with approximately the following frequency: nerve fiber layer, in 10 per cent of the cases; inner nuclear layer, in 60 per cent; outer nuclear layer, in 20 per cent, and both the inner and the outer layer, in 10 per cent. It is therefore with the constituents of the inner and outer nuclear (granular) layers that glioma is principally concerned. Since neuro-epithelioma is so primitive histogenetically that differential metallic staining is difficult (or at times apparently impossible), a tumor composed of adult astrocytic, oligodendroglial or ependymal cells, which stain readily with these methods, may henceforth be excluded from immediate differential consideration.

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9. Parsons, J. H.: The Pathology of the Eye, New York, G. P. Putnam's Sons, 1905, vol. 2, p. 636.

Ocular Neuro-Epithelioma.—Flexner,[5] in his description of the histologic picture in the first recognized case of neuro-epithelioma of the retina, stated that the acute ends of the cells forming the characteristic rosettes projected beyond the cuticular membranous border into the lumen of the rosette, in a manner similar to that in which rods and cones project beyond the membrana limitans externa. Not only was colloid or hyaline material often present in the lumen, "apparently . . . from a gradual transformation of the rod and cone structures," but "in certain rosettes the processes are quite wanting, again they are present in rudimentary form, consisting of knob-like projections, and finally they can be recognized as distinct prolongations. A process similar to this gradual change is seen in the embryo in those animals in which the development of rods and cones is incomplete at birth, and it is described by Hertwig [10] as follows: 'In all vertebrates, so long as the rods and cones are not present, the inner layer of the optic cup is separated from the outer by a complete smooth contour proceeding outward from the membrana externa. Later there appears upon this membrane numerous small, refractive knob-like processes that have been produced from the peripheral ends of the external nuclear or visual cells. These knobs consist of a protoplasmic material that stains in carmine; they extend somewhat lengthwise and form the internal segments of the rods and cones. Finally there appears on their surface the external segments, the lamellated structure of which led Max Schultze and W. Müller to compare them to cuticularization.'" Flexner also noted that the tumor showed bands of connective tissue and that the interstitial cells between the rosettes proper "compared with those of the external nuclear layer . . . [as to] form, size and staining affinity." Much of the tumor which he described, however, showed only perivascular viability, and there was a "deposit of lime salts in the degenerated portions."

Although much neuropathologic tissue was formerly designated as neuro-epithelioma, the very existence of the actual cerebral neoplasm has been questioned within the past decade. Bailey and Cushing [11] stated that the primitive spongioblast is the essential stem cell of the tumor and described the tissue as a cellular, fairly well vascularized neoplasm tending to form groups of cells with small canals or cavities as rosettes which do not have a limiting basement membrane but do have a cuticular membrane lining the canaliculi. The columnar cells of the rosettes have blepharoplasts aggregated in a row at the free border, and if fresh they may show cilia; the long process at the pole opposite the blepharoplasts is difficult to demonstrate because it stains neither as neurofibrillae nor as gliofibrillae. The tissue intervening between the rosettes resembles medulloblastoma in architecture, and the tumor has an abundant reticular network.

10. Hertwig, Oscar: Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbelthiere, Jena, Gustav Fischer, 1888, p. 357.

11. Bailey, P., and Cushing, H.: A Classification of the Tumors of the Glioma Group on a Histogenic Basis with a Correlated Study of Prognosis, Philadelphia, J. B. Lippincott Company, 1926.

The striking characteristic of the cerebral tumor is that it forms true rosettes. In the retinal tumor the cells of the rosettes bear a single cilium with a diplosome at its base,[12] and the inner ends of the cells not infrequently project into the lumen beyond the internal limiting cuticular membrane,[13] as do rods and cones. If the analogy between the cerebral and the retinal tumor is regarded as substantially valid, the systemic place of the cerebral tumor seems to depend on whether the retinal rods and cones are regarded as neuronal or as glial tissue. Investigators have customarily begged the question by calling them merely epithelial; hence the term "neuro-epithelioma."[14] However, primitive spongioblasts in the olfactory bulb also have only a single diplosome[15] and so have the cells of Müller in the retina, unlike similar cells elsewhere in the developing brain (where primitive spongioblasts bear several cilia and numerous diplosomes). If regarded in this light, the tumor constituent is primitively glial. This corresponds better in general with the nuclear and cytoplasmic characteristics of the cell, though even so it stains only indifferently with gold sublimate or the Golgi silver chromate methods.

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12. Mawas, J.: Bull. Assoc. franç. p. l'étude du cancer **13**:78, 1924.

13. Verhoeff, F. H.: Roy. London Ophth. Hosp. Rep. **16**:309, 1903.

14. Flexner[5] stated: "Embryology has now shown us that the two external layers of the retina are not to be considered as purely nervous. It is believed that in the course of development [Foster, M., and Balfour, F. M.: The Elements of Embryology, ed. 2, New York, Macmillan & Co., 1883, p. 145. Schwalbe, G.: Lehrbuch der Anatomie der Sinnesorgane, Erlangen, E. Besold, 1887, p. 93] the retina becomes divided into an outer part corresponding to the epithelial lining of the cerebrospinal canal, yielding what may be called the visual cells (*Sehzellen*, W. Müller) of the eye, i. e., the cells forming the outer nuclear layer, and the layer of rods and cones attached to them, and an inner part consisting of the remaining layers of the retina, including the nerve fibers, which correspond morphologically, to the substance of the brain and spinal cord. Kölliker [von Kölliker, A.: Entwicklungsgeschichte des Menschen und der höheren Thiere, Leipzig, Wilhelm Engelmann, 1879, vol. 2, p. 693] regards the rods and cones as cuticularizations of the cells destined to form the external nuclear layer, while Schwalbe [Lehrbuch der Anatomie der Sinnesorgane, Erlangen, E. Besold, 1887, pp. 92 and 83] states that the retina can be divided into two parts consisting of the cerebral portion of W. Müller (*Gehirnschicht* of Schwalbe) and the epithelial layer. The latter Schwalbe denominates neuroepithelium, and it comprises the layer of external nuclei, the external limiting membrane and the layer of rods and cones, and finds its analogue in the epithelial cells of taste and of smell. . . ." It was by this reasoning that Flexner was led initially to denominate the retinal tumor neuro-epithelioma, a name now firmly entrenched but unfortunate in the breadth of its application, for even paraphysial cysts (McLean, A. J.: Arch. Neurol & Psychiat., to be published) have been designated, by the suggestion of an eminent neurohistologist (Rinder, Carl O., and Cannon, Paul R.: Arch. Neurol. & Psychiat. **30**:880, 1933) as "neuro-epithelial cysts."

15. Leboucq, G.: Arch. d'anat. micr. **10**:555, 1909.

Nuclear Characteristics of the Present Cerebral Neuro-Epithelioma.—In view of the uncharacteristic cytoplasmic detail of our tumor and our mediocre success with specific impregnation methods, the nuclear structure of the tumor cells was studied in greater detail, in an endeavor to homologize it with that of retinal cells of known function. The nuclei fell roughly into two groups: (1) a large vesiculiform nucleus with a fine chromatin network and a single well formed solid nucleolus and (2) a slightly smaller nucleus, with heavier membrane and chromatin aggregated into from two to six slightly irregular, heavy globules strung at the junctions of linin cords. The latter type was predominant in the cells comprising the rosettes; such nuclei resembled closely the nuclei of amacrine cells and also those of certain medulloblastomas. Their resemblance to the nuclei of amacrine cells was of small assistance, however, for as noted, the glial or neuronal nature of these cells is still unsettled. The vesicular neuroblastic nucleus was observed predominantly in the filler-in cells between the rosettes, canaliculi and other architectural formations of the tumor; it was the nucleus of the cells of the broad pavement sheets already described. Neither nuclear type, however, was wholly sharply segregated; possibly from 5 to 10 per cent of the rosette cells contained "neuroblastic" nuclei, and there were both scattered cells and nests with the "glial" nuclei present in the polygonal cells of the filling material. But by no stretch of the imagination could the nuclei of either cell group in our tumor be considered to be analogous to those of the normal external granular layer of the retina, though all the other criteria presented by Flexner were satisfied.[16]

Both the nuclear types present in the tumor resembled greatly those found in primitive medullary epithelium, and if an external limiting membrane were definable anywhere in our tumor (and if the cilia and blepharoplasts were absent) its predominant architecture of tubular and acinar formation and of folding would make tenable a histologic diagnosis of the even more rare medullo-epithelioma.[17] All the nuclei shown in Tello's[3] illustrations are similar to only the second type in our tumor. Tello was able to demonstrate not only abundant primitive spongioblasts but astroblasts and astrocytes; his illustrations of the neuroblasts observed are not wholly convincing, however. In our tumor no spongioblasts could be demonstrated by specific methods during repeated trials, but, on the other hand, "glial" nuclei were noted in abundance, as were also great numbers of "neuroblastic" nuclei, and it was possible to stain specifically the fibrils in the tails of a few of the neuroblastic cells.

The name neuro-epithelioma has been based on the postulation that the rosette cells are analogous to rod and cone cells, and though many points support this (including the fact that our tumor had its source

16. It should be pointed out that Flexner's tumor was considerably degenerated and that the tissue was fixed in alcohol (notoriously poor for nuclear study when employed in less than absolute strength). He admitted that "owing to the manner of preserving the tissue the finer details could not be worked out."

17. Fay (Arch. Neurol. & Psychiat. **26**:674, 1931) has reported a case of primitive cerebral tumor the cytologic characteristics of which were obscure. Although Winkelman diagnosed the tissue as medullo-epithelioma, the published histologic description is meager. Despite the fact that the tumor was "certainly glial in origin," it developed in the discussion that it was of "distinctly neuroblastic type." Alpers, through whose hands passed the original tissue taken (by Frazier) for biopsy diagnosed the material as neuroblastoma (personal communication to the author, Aug. 8, 1935).

near the site of origin of the primitive optic vesicle), the nuclei observed in the present tumor and those shown in illustrations of the histologic details in other cases of neuro-epithelioma do not even faintly resemble those of the normal external granular layer of the retina. Moreover, even if the analogy to rod and cone cells were complete, the systematic placement of the tumor would not be much furthered, for rod and cone cells are at present recognized neither as glial nor as neuronal and, furthermore, cannot be shown to homologize with primitive spongioblasts.

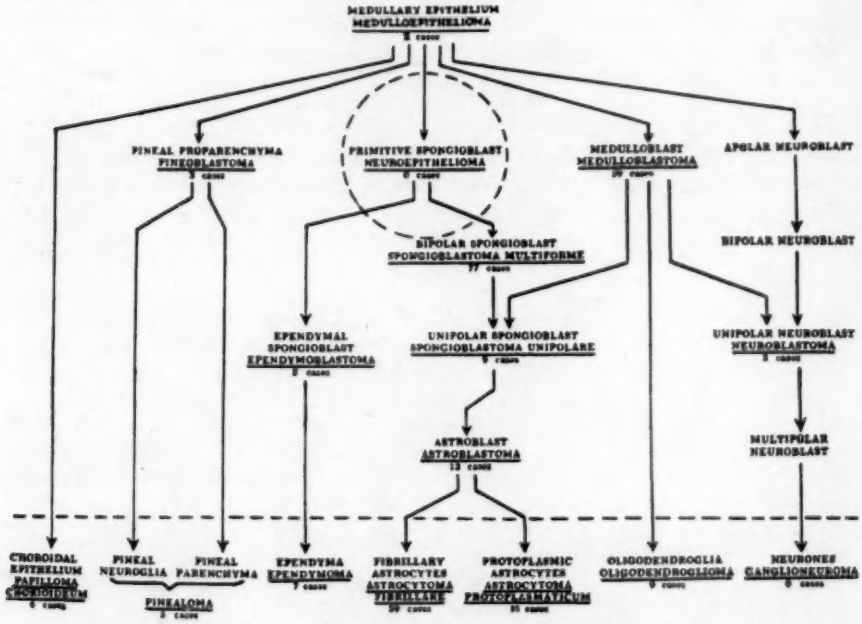

Fig. 4.—The Bailey and Cushing [11] schema for the classification of cerebral gliomas, in which they are coordinated with cells occurring in the normal histogenesis of the central nervous system.

SYSTEMIC SCHEMATA

The glial tumor described in this paper, containing, as it did, considerable amounts of tissue with indubitable neuroblastic nuclei, falls astraddle fundamental histogenetic barriers in any promulgated comprehensive schema of cerebral parenchymal tumors. The schema of Bailey and Cushing (fig. 4) provides for an analogous difficulty in the case of the medulloblastoma by showing the medulloblast (adendritic indifferent cell of Schaper) as pluripotential in development, and the authors provided good histologic substantiation for this attribute,[18] but

18. Bailey and Cushing,[11] p. 58.

the schema denies pluripotentiality to the primitive spongioblast and shows it to be capable of development only into polar spongioblasts or ependymoblasts. It should be said parenthetically that at this time (1926) no neuro-epitheliomatous tissue was included in their series and that of the two cases which were reported six years later,[1] the description of one was not critically interknit with the literature and in the other the correctness of the placement of the tumor was not wholly certain.

One is faced with an augmented but similar difficulty in an attempt to fit our tumor into the Hortega schema (fig. 5), the retinal tumor with rosettes being shown in this classification to be of sole neuroblastic origin [19] and the glial tumor of presumed "neuro-epitheliomatous" type being included under the omnibus heading of glioblastoma (fig. 5*c*), which derives its origin from glioblasts having mere tripotentiality. Hortega admitted minor "participation of neuroglial and connective tissue elements" to the category of retinal neuroblastoma. His systematization throughout is considerably less specific than that of Bailey and Cushing, and although this may possibly be more in accord with the confusing neoplastic actuality at times, it probably begs the orderly specificity which strict histogenesis ultimately implies.

The classification of Roussy and Oberling [20] is based on pure morphologic characteristics; it has gained little adherence outside of France and has served further to confuse an already obscure picture.

Orographic architecture in some degree is not uncommon to many primitive tumors. About a third of the actual rosettes in our tumor showed no blepharoplasts or cilia; this, in well preserved tissue, is capable of an interpretation other than that of technical fault in staining. Bailey and Cushing drew attention to such unusual rosettes in the single case of medullo-epithelioma [21] which they reported. If our tumor were to be considered as a medullo-epithelioma, many systematic difficulties would vanish, for the pluripotentiality of its components would then allow simultaneous differentiation both toward primitive spongioblasts and toward neuroblasts. The presence of both types of cells could be explained and the incompletely differentiated rosettes accounted for. Against this would remain the mere fact that a basement membrane was nowhere demonstrable in our tumor. The Bailey and Cushing classification, however, is based on the eminently sensible foundation that the name and the behavior of the tumor are determined by the predominant type of tissue present in the mass. In the case of the

19. "No true tumors of the neuroblastic series have been described" in the retina (Grinker, R. R.: Arch. Ophth. **5**:920, 1931).

20. Roussy, G., and Oberling, C.: Atlas du cancer, Paris, Félix Alcan, 1931.

21. Bailey and Cushing,[11] p. 28.

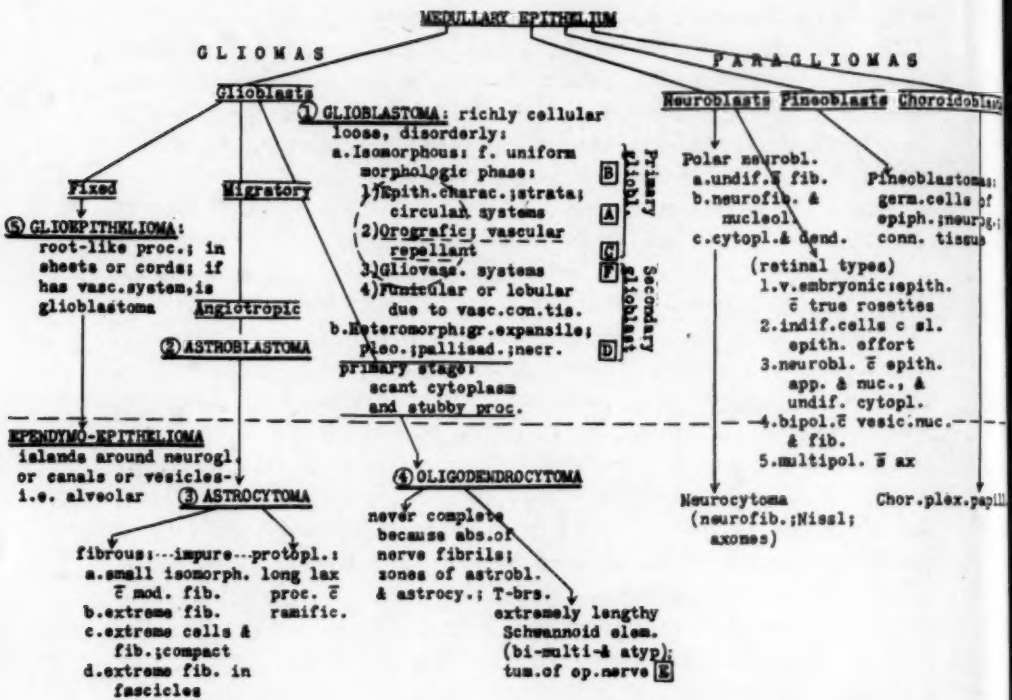

Fig. 5.—Histogenesis of tumors of the central nervous system, after Hortega (del Rio-Hortega, P.: Arch. españ. de oncol. **2**:411, 1932), based solely on silver carbonate staining methods. It will be observed that it differs from the schema of Bailey and Cushing (fig. 4) principally in the nonacceptance of the medulloblast, the terminologic repudiation of the well entrenched spongioblast and the apparent aggregation of many clinically distinctive tumors under the omnibus heading of glioblastoma. Hortega expressed the belief that the indifferent adendritic cell of Schaper (Schaper, A.: Arch. f. Entwcklngsmech. d. Organ. **5**:81, 1897) is not bipotential and is the progenitor of oligodendroglia; the tumor generally known as spongioblastoma polare seems thus to become a special differentiation of oligodendroglioma. In an attempt to dissect Hortega's heterogeneous grouping "glioblastoma," I have indicated by letters in squares my interpretation of his subvarieties in terms of the classification of Bailey and Cushing, thus: *A* denotes medullo-epithelioma; *B*, medulloblastoma; *C*, neuro-epithelioma; *D*, glioblastoma multiforme; *E*, spongioblastoma polare, and *F*, astroblastoma. However anguished the histologic task occasionally may be, clinical experience and prognosis warrant the use not of subvarieties but of types; e. g., tumors of types *B* and *D* differ clinically not only in their cerebral location but in the age group in which they occur, and for type *F* the average life expectancy is at least four times that for either type *B* or type *D*.

tumor described here, it is utterly impossible to deny that the major bulk of tissue examined was indubitably neuro-epitheliomatous and that the tumor should remain so classified. It is also equally impossible to escape the fact that such a designation does violence not only to schematization but to the total structure of the tumor.

That the name neuro-epithelioma is particularly unfortunate has been discussed by others;[22] the designation was originally based on a then helpful but fancied resemblance to retinal rod and cone cells, which in many characteristics the tumor cell does not even faintly resemble. The name suggested as an alternative, spongioblastoma, has many disadvantages.[23] Neurospongioblastoma or spongioblastoma neuromatosum in our case would constitute merely a descriptive dialectic straddle, without aiding systematic clarity. Until a sufficient number of careful descriptions of authentic tumors of this type are accumulated in the literature or until a comprehensive study of registered material and garnered specimens can be undertaken by such an agency as the Brain Tumor Registry at Yale University, it probably is best reluctantly to retain for present usage the designation in the Bailey and Cushing classification, for, as Cox[24] has pointed out, this scheme "embraces names that have been widely accepted in the English-speaking world . . . [and] has the great advantage of being the common language of many neurologists, neurosurgeons and pathologists."

SUMMARY

A rare primitive cerebral tumor is reported. The bulk of its tissue was observed to be indubitably neuro-epitheliomatous, but it contained a generous admixture of neuroblasts. Its cytologic characteristics and systemic classification are discussed.

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22. Bailey and Cushing,[11] p. 95.

23. Bailey, P.: Bull. Johns Hopkins Hosp. **40**:354, 1927.

24. Cox, L. B.: Am. J. Path. **9**:839, 1933.

EFFECT OF ALCOHOL ON CHOLESTEROL-INDUCED ATHEROSCLEROSIS IN RABBITS

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There has been an impression among pathologists for many years that confirmed drunkards do not show as much arteriosclerosis for given age groups as do temperate persons. Whether the cause for this difference is a direct chemical effect of the alcohol in the blood or whether it is the effect of varying amounts of cholesterol in the diet is not known. Martland [1] stressed the fact that there are great differences in activity and diet both between persons with chronic alcoholism and temperate persons and between two large groups of persons with chronic alcoholism. He suggested that these differences may be of more importance in the degeneration of the person than the actual presence of the alcohol in the blood. On the other hand, Turner,[2] Turner and Khayat,[3] Page and Bernhard [4] and others have shown experimentally that iodine has a profound effect on the response of rabbits fed cholesterol, thus demonstrating a direct chemical action on the cholesterol metabolism and indicating the possibility that alcohol can have a similar effect. Probably both mechanisms will be found to play a rôle in arteriosclerosis in man. The present work presents some data on the effect of alcohol per se on the rabbit fed cholesterol.

METHOD

Twenty gray chinchilla rabbits of both sexes and between 5 and 6 months old were used, being divided into four groups of five each. All were kept on a basic diet of oats and alfalfa, with the addition once a week of one leaf of cabbage and one small piece of carrot. Cholesterol was obtained by extraction with ether and recrystallization of gallstones with a mixture of ether, alcohol and water and was administered in crystalline form mixed with the oats, according to the method of Turner.[2] The alcohol was given as a 25 per cent solution of a 95 per cent concentration of commercial grain alcohol in water. The rabbits refused to drink this at first and had to be educated over a period of about three weeks by the use of concentrations which were increased slowly from 1 per cent. A rough check

From the laboratories of Surgical Pathology of the College of Physicians and Surgeons and the Presbyterian Hospital.

1. Martland, H.: The Pathology of Chronic Alcoholism, in Emerson, H., and others: Alcohol and Man, New York, The Macmillan Company, 1932, chap. 9, p. 201.
2. Turner, K. B.: J. Exper. Med. **58**:115, 1933.
3. Turner, K. B., and Khayat, G. B.: J. Exper. Med. **58**:127, 1933.
4. Page, I. H., and Bernhard, W. G.: Arch. Path. **19**:530, 1935.

was kept on the amount of alcohol consumed by each animal by the use of 100 cc. drinking tubes, and it was found that the maximum intake occurred at a concentration of 25 per cent, with an average of 100 cc. of solution per day, or about the alcohol equivalent of an imperial quart (1,136 cc.) of 100 proof whisky per day for a man weighing 68 Kg.

Group 1 (animals 1 to 5, inclusive) received the basic diet plus alcohol.

Group 2 (animals 6 to 10, inclusive) received the basic diet plus 0.5 Gm. of cholesterol per day and alcohol.

TABLE 1.—*Determinations of the Cholesterol Content of the Blood* *

	Group 1					Group 2					Group 3					Group 4				
Rabbit 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Day																				
0	...	...	...	...	...	...	...	...	...	...	72	122	151	...	...	...	...	...	...	...
1	...	...	...	...	...	...	...	...	...	...	...	...	...	109	99	124	130	103	121	112
5	127	146	149	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
6	...	...	...	124	158	131	140	124	123	...	...	...	...	...	...	...	...	...	...	...
7	...	...	...	...	...	...	...	...	...	137	117	114	94	134	117	...	...	...	...	...
8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	117	134	...	120	...
9	109	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	90	...	91
12	...	116	109	97	119	92	113	...	...	...	...	...	...	...	...	...	...	...	...	...
13	...	...	...	...	...	...	...	117	108	94	88	83	121	85	78	...	...	...	...	...
14	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	112	132	99	103	73
15	133	134	109	84	125	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
18	...	...	...	...	...	143	126	97	106	75	...	...	...	...	...	...	...	...	...	...
21	Cholesterol feeding started																			
40	134	128	135	116	159	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
41	...	...	...	...	...	647	374	434	416	185	...	...	...	...	...	...	...	...	...	...
44	...	...	...	...	...	...	...	...	...	...	229	352	370	162	86	100	106	†	...	...
54	108	162	164	89	131	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
55	...	...	...	...	...	‡	378	444	598	529	...	...	...	...	...	...	...	...	...	...
61	...	...	...	...	...	...	...	...	...	...	200	352	231	133	90	...	...	...	...	...
63	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	90	107	...	90	83
70	124	135	154	107	109	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
72	...	...	...	...	...	...	564	613	604	257	...	...	...	...	...	108	121	...	114	95
75	...	...	...	...	...	...	...	...	...	...	194	239	359	109	...	...	...	...	...	...
84	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	106	117	...	125	105
85	142	157	...	116	122	...	812	958	664	513	...	...	...	...	...	...	...	...	...	...
89	...	...	...	...	...	...	...	...	...	...	375	593	552	327	254	...	...	...	...	...
103	140	160	131	123	162	...	...	...	...	...	...	...	...	...	...	130	...	...	...	...
110	...	...	...	...	...	...	804	846	662	355	...	...	...	...	...	...	113	...	...	99
111	...	...	...	...	...	...	...	...	...	...	484	597	...	...	...	...	...	...	...	...
117	...	...	...	...	...	...	...	...	...	...	...	...	497	250	156	...	...	100	100	...
124	137	99	141	117	111	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
128	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	66	89	84	56	...
134	...	...	...	...	...	...	874	712	708	321	...	...	...	...	...	93	...	...	76	73
138	...	...	...	...	...	...	...	...	...	...	512	404	414	250	...	...	...	...	...	...
160	156	125	196	107	117	...	...	...	...	...	...	...	...	...	...	...	139	114	93	80
161	...	...	...	...	...	...1,136	857	574	605		905	774	905	142	438	...	...	...	...	...

* The readings are in milligrams per hundred cubic centimeters of blood.
† A litter of six was born. The animal was not used for sixty-nine days.
‡ The animal broke its neck struggling in the bleeding box. A determination made post mortem was 547 mg.

Group 3 (animals 11 to 15, inclusive) were fed the basic diet plus 0.5 Gm. of cholesterol per day but were not given alcohol.

Group 4 (animals 16 to 20, inclusive) were kept as controls on the basic diet and water.

Determinations of the cholesterol content of the blood were made by the method of Myers and Wardell [5] on the entire set of animals three times before feeding of cholesterol was started and as often thereafter as time permitted. The results for individual animals are given in table 1, and the averages for the group for each set of determinations, in table 2. Figure 1 presents the data from table 2 in graphic form.

5. Myers, V. C., and Wardell, E. L.: J. Biol. Chem. **36**:147, 1918.

Determinations of the alcohol content of the blood were also made on all animals once and on the groups fed alcohol several times, the method of Gettler and Freireich[6] being used, but no appreciable rise above the normal titratable acidity was found at any time.

At the end of one hundred and sixty-two days the animals were killed; autopsies were performed, and the heart, aorta, liver, one kidney and a section of the femoral vessels were placed in a dilute solution of formaldehyde U. S. P. (1:10).

Determination of the Cholesterol Content of the Liver.—The method for determining the cholesterol content of the liver is as follows: The liver was weighed while wet, and three small portions were cut out for analysis. Each of these was weighed accurately on a gravimetric balance, and one of them was placed

TABLE 2.—*Values for the Cholesterol Content of the Blood by Groups*

Day	Group 1 Normal Diet Plus Alcohol	Group 2 Normal Diet Plus Alcohol and Cholesterol	Group 3 Normal Diet Plus Cholesterol	Group 4 Normal Diet (Control)
0	...	...	110	...
1	...	...	...	118
5	141	...	...	...
6	...	131	...	...
7	...	...	115	...
8	...	...	...	110
12	110	...	...	...
13	...	165	91	...
14	...	...	...	104
15	121	...	...	...
18	...	110	...	...
40	134	...	...	...
41	...	411	...	...
44	...	...	240	...
54	131	...	...	...
55	...	504	...	...
61	...	...	201	...
70	126	...	...	...
72	...	509	...	110
75	...	...	225	...
84	...	...	...	113
85	134	716	...	...
89	...	...	420	...
103	143	...	...	...
108	...	...	...	114
110	...	583	...	...
113	...	...	396	...
123	121	...	...	92
133	...	653	...	...
138	...	...	397	...
160	140	...	...	107
161	...	793	633	...

in a drying oven at a temperature of 100 C. and dried to constant weight. The other two were cut into strips about 1 mm. thick and transferred quantitatively to 250 cc. Erlenmeyer flasks with 100 cc. of a 3 per cent solution of potassium hydroxide in a 40 per cent concentration of alcohol. After twenty-four hours at room temperature these were heated on a steam bath until solution of tissue was complete (within from two to four hours). They were then cooled; the concentration of alcohol was raised to approximately 70 per cent, and extraction with ether was carried out four times, from twenty-four to forty-eight hours being allowed for the first extraction and from twelve to twenty-four hours for each of the other three. These ether fractions were then washed with distilled water until they were clear and neutral to litmus; the washings were extracted once with ether

6. Gettler, A. O., and Freireich, A. W.: J. Biol. Chem. **92**:129, 1931.

and the combined extracts were made up to the desired volume. A volume of 100 cc. was used for the groups not given cholesterol (1 and 4), and a volume of 200 cc., for the groups given cholesterol (2 and 3). The determination of the cholesterol content was carried out on aliquot parts of these extracts by the method of Bloor, Pelkan and Allen,[7] and the results were calculated in grams per hundred grams of dry weight. The dry weights had previously been calculated

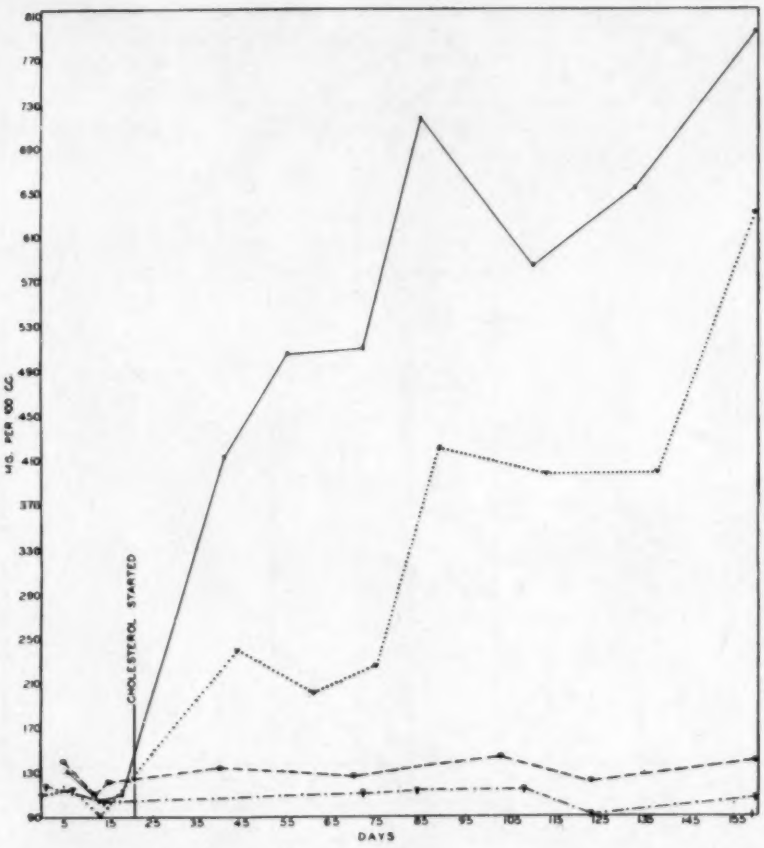

Fig. 1.—Graphic presentation of the cholesterol content of the blood for the four groups. The broken line indicates the group fed alcohol but no cholesterol (group 1); the solid line, the group fed alcohol and cholesterol (group 2); the dotted line, the group fed cholesterol but no alcohol (group 3), and the line of dashes and dots, the control group (group 4).

from the percentage found for the dried section. In table 3 will be found the averages of the duplicate samples of each liver. Figure 2 shows the average concentration of cholesterol in the livers of each group.

7. Bloor, W. R.; Pelkan, K. F., and Allen, D. M.: J. Biol. Chem. **52**:191, 1922.

Determination of the Cholesterol Content of the Aorta.—The estimations of cholesterol in the aorta were made by Dr. Warren M. Sperry. The aorta was separated from the heart just above the valves and was cleaned carefully of all adventitia, as recommended by Schoenheimer.[8] A small section was taken from each arch and one from the region of the superior mesenteric artery for microscopic examination. The remainder of the aorta was placed in a drying oven at a temperature of 100 C. for one hour, and in a vacuum desiccator until dried to constant weight (from four to five days) and then was transferred quantitatively to 125 cc. flasks. It was left for twenty-four hours at room temperature in 50 cc. of a

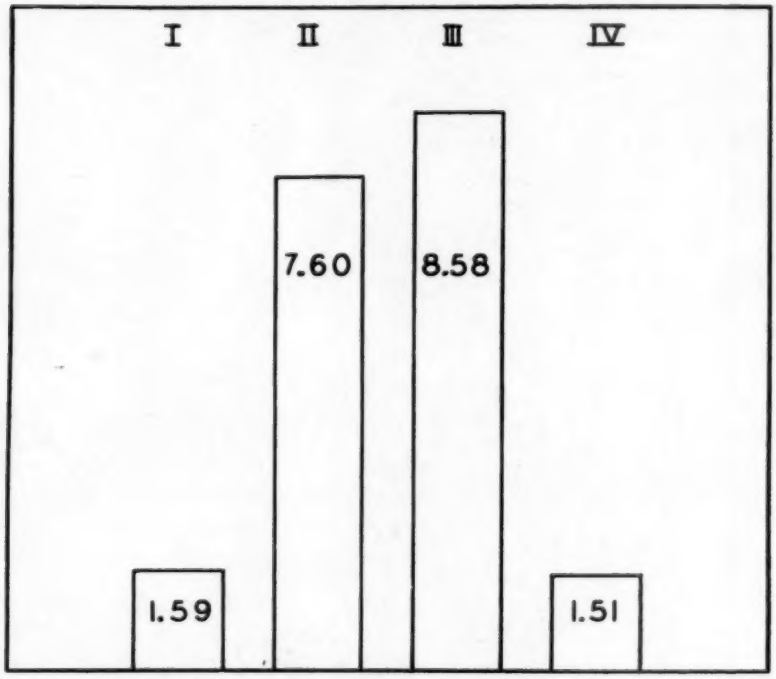

Fig. 2.—Averages of the cholesterol content of the liver for the four groups.

4 per cent solution of potassium hydroxide in an 80 per cent concentration of alcohol and heated on a steam bath for four hours, after which extraction with ether was carried out as with the sections of liver. Again the determination method of Bloor, Pelkan and Allen was tried, but an unsaponifiable substance, soluble in ether and chloroform, was found to be present, which formed a bluish-purple cloud on the addition of the sulfuric acid in the Liebermann-Burchard reaction. All attempts to remove this substance proved completely futile with the technic of Bloor, Pelkan and Allen, but the micromethod of Schoenheimer and Sperry[9] was found to be satisfac-

8. Schoenheimer, R.: Ztschr. f. physiol. Chem. **160**:61, 1926; **177**:143, 1928.

9. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:745, 1934.

tory and reliable.[10] The results, as shown in table 3, were calculated on a basis of grams per hundred grams of dry weight.

Frozen sections and sudan III stains of all the specimens were made for histologic study.

COMMENT

Chemical Analysis.—Cholesterol Content of the Blood: Figure 1 shows a rapid rise in the cholesterol content of the blood of the group fed alcohol and cholesterol to a level almost twice as high as that of the group fed cholesterol but no alcohol, and then a terminal slowing, while the level of the group not given alcohol tends to rise more rapidly. These observations bring up several points of interest. One is that the appetites of the rabbits fed alcohol (group 2) became so impaired

TABLE 3.—*Cholesterol Content of Livers and Aortas*

		Liver			Aorta		
Group	Animal	Wet Weight of Whole Liver, Gm.	Dry Weight, Percentage of Wet Weight	Cholesterol, Gm. per 100 Gm. of Dry Weight	Dry Weight, Gm.	Cholesterol, Gm. per 100 Gm. of Dry Weight	Gross Evidence of Atherosclerosis
1	1	63.9	26.5	1.18	0.2222	*	None
	2	86.2	23.0	1.22	0.2167	0.49	None
	3	72.7	25.9	1.72	0.1889	0.36	None
	4	69.5	26.4	1.49	0.2580	0.21	None
	5	54.5	28.2	2.33	0.2330	0.29	None
2	6	Died on fifty-fifth day					
	7	91.8	29.2	7.23	†	....	Moderate
	8	81.7	29.9	8.80	0.2134	1.31	Marked
	9	77.2	28.2	7.49	0.1920	1.96	Marked
	10	92.5	29.0	6.88	0.1759	0.52	Slight
3	11	123.6	33.2	10.84	0.1596	2.66	Marked
	12	106.9	32.9	12.99	0.2444	1.40	Marked
	13	115.7	28.1	6.49	0.2211	4.36	Marked
	14	116.0	29.8	7.58	0.2044	1.18	None
	15	101.5	29.4	4.08	0.1027	0.39	None
4	16	63.0	26.2	1.61	0.0984	0.93	None
	17	63.5	30.3	1.45	0.1468	0.71	None
	18	75.7	25.5	1.61	0.1458	0.59	None
	19	75.0	25.6	1.65	0.1895	0.73	None
	20	72.7	24.2	1.22	0.0987	0.84	None

* All the extract was used in attempting to purify for the Liebermann-Burchard reaction.
† The aorta was lost between the time of autopsy and the time of analyses.

after a few weeks that they ate only about three daily rations of grain per week. As no cholesterol was added to their portions to make up this deficit, their actual ingestion of cholesterol was about half that of the rabbits that were not given alcohol (group 3); yet in spite of this low intake their blood cholesterol level rose to the greater height and remained there over a considerable period. Whether the terminal approach of the values for the two groups was permanent or was one

10. These results, as well as those of the determinations on the livers, may well be questioned on an absolute basis because of the use of formaldehydized tissue (note the difficulty encountered in determining the cholesterol of the aorta), but as all the specimens were subjected to exactly the same technic I think that the results may be considered reliable as relative values.

of the cyclic regressions typical of animals fed cholesterol cannot be known. However, Turner and Bidwell [11] have found in their work on iodine that a time comes when all protective mechanisms break down in the face of the cholesterol flood, and the possibility arises here that a blood cholesterol-sustaining substance may also be limited in its effectiveness. It will also be noted in table 1 that rabbit 10, an animal given alcohol, and rabbit 14, an animal not given alcohol, were resistant to cholesterol; this is a disturbing phenomenon encountered occasionally in all strains of rabbits. Rabbit 15 also showed no rise in the cholesterol

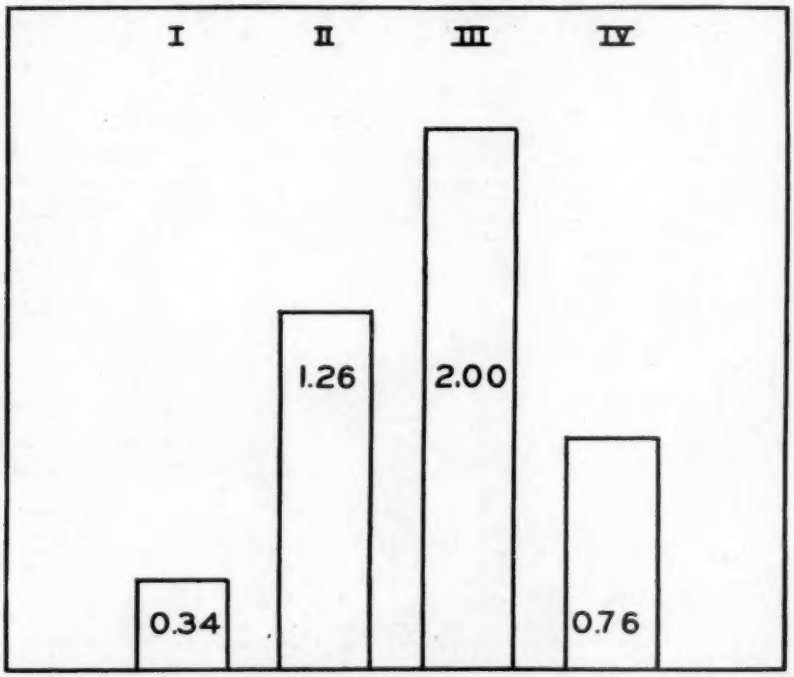

Fig. 3.—Averages of the cholesterol content of the aorta for the four groups.

content of the blood, but this was because he "shucked" his oats and refused to eat the cholesterol-contaminated husks. The slight elevation of the cholesterol content of the blood in group 1 (given alcohol) above that of group 4 (the controls), both of which groups received no cholesterol, is so slight as to be without great significance in itself but is constant and becomes of interest in relation to the cholesterol content of the aorta in these groups, as will be brought out later.

Cholesterol Content of the Liver and Aorta: In table 3 and figures 2 and 3 one observes a relationship between the cholesterol content of

11. Turner, K. B., and Bidwell, E. H.: J. Exper. Med. **62**:721, 1935.

the liver and of the aorta of the animals given alcohol and cholesterol and that of those given cholesterol but no alcohol, which is the reverse of the levels of the cholesterol of the blood, the content of group 2 (given alcohol) being lower than that of group 3 (not given alcohol). It is possible, of course, that the difference in the intake of cholesterol is responsible for the low lipoid content of the tissue, but I believe that this is improbable for the following reasons: It is known [12] that cholesterol is deposited in both the liver and the aorta to a large degree as combined cholesterol. This means that the free cholesterol eaten must be metabolized somewhere (liver or gastro-intestinal tract?) and transported to the aorta in the blood stream. Yet the cholesterol level of the blood of group 2, the animals which were given alcohol and which ate little cholesterol, rose higher than that of group 3, while the cholesterol content of the tissues remained lower. A low intake alone cannot explain both these findings. The difference in the cholesterol content of the liver between groups 1 and 4, which received no cholesterol, is negligible, but the difference in the cholesterol content of the aorta is large enough to be significant and becomes of greater interest because the relation between the cholesterol content of the blood and that of the aorta of these groups is seen to be the same as that between the two groups fed cholesterol.

So it would appear that alcohol per se may have some effect on both the cholesterol-metabolizing and the cholesterol-synthesizing mechanism of rabbits in the way of causing more cholesterol to circulate in the blood stream but in some form or under such conditions as to prevent it from being absorbed into the tissues, though there are, of course, several objections that may be raised to this analysis. Statistically, "samples" as small as my groups of from three to five animals are too small to give valid results. Further, it will be seen in tables 1 and 3 that only in the determinations of the cholesterol content of the aortas of groups 1 and 4 are there no overlapping values. For example, if the aberrant rabbits 14 and 15 are eliminated from group 3, the cholesterol levels of the blood of the group given alcohol and of the group fed cholesterol but not alcohol come closer together throughout, and in the last determination the cholesterol level of the group not given alcohol rises above that for group 2. Eliminating the data for these animals from tables showing the cholesterol content of the liver and the aorta merely accentuates the differences already present. I think that one may safely state that the two significant findings are the maintenance under the influence of alcohol of a high cholesterol content of

12. Meeker, D. R., and Jobling, J. W.: Arch. Path. **18**:252, 1934. Sperry, W. M., and Stoyanoff, V. A.: J. Nutrition **9**:131, 1935. Schoenheimer.[8]

the blood on a low intake of cholesterol and the lowering of the normal cholesterol content of the aorta. But I do not think that these findings are necessarily final or that any dogmatic conclusions should be drawn from them.

Morphologic Analysis.—General: During life no essential differences were noticed between the animals of the four groups except for the poor appetites and slight lethargy of the animals given alcohol. At autopsy the rabbits fed cholesterol, both of the group given alcohol and of the group not given alcohol, showed definitely more depot fat.

Livers: Between the livers of the animals given alcohol and those of the animals given neither alcohol nor cholesterol (groups 1 and 4, respectively), no gross differences could be seen. Both groups had small, dark red, essentially normal livers. Microscopically, too, no definite deviation from the normal was seen except for a few small focal deposits of fat in some of the animals not given alcohol which were not seen in the animals given alcohol. Both groups that were fed cholesterol presented grossly large, pale yellow livers with tense capsules, rounded edges and greasy cut surfaces. Microscopically, there were in both groups extensive, severe vacuolation and replacement of the cytoplasm of liver cells by fat, as well as loading of the Kupffer cells with lipoid. This process was more marked in the central zones, a localization more noticeable in the animals given alcohol and in line with their lower cholesterol content. A section of the liver from rabbit 3, an animal that received alcohol but no cholesterol, is shown in figure 4 *B*, and representative sections from an animal given alcohol and cholesterol and from an animal fed cholesterol but no alcohol are shown in figures 4*E* and *F*. No lipoid is seen in bile ducts, nor is there any histologic evidence of cirrhosis or other damage to the liver.

Aortas: Figure 5 shows the gross appearance of the aortas, those above (*7* to *15*) belonging to the two groups fed cholesterol, and those below (*1* to *5* and *16* to *20*), to the two groups not fed cholesterol. Atheromatous plaques are seen in three aortas of each of the groups fed cholesterol (*7*, *8* and *9*, and *11*, *12* and *13*) but in none of the groups not fed cholesterol. A few were present in the aorta of rabbit 10 but do not show in the photograph. However, it is plain that the differences found in the cholesterol content of the aortas chemically do not show in any striking fashion as gross pathologic changes. In the fresh specimens the plaques in the animals given alcohol appeared slightly smaller and were more diffusely distributed, and the intervening tissue was more translucent. Microscopically, as is seen in figure 4 *C* and *D*, the plaques from the aortas of the animals given alcohol did not show the large "blobs" of lipoid or as much invasion of the media as those from animals not given alcohol.

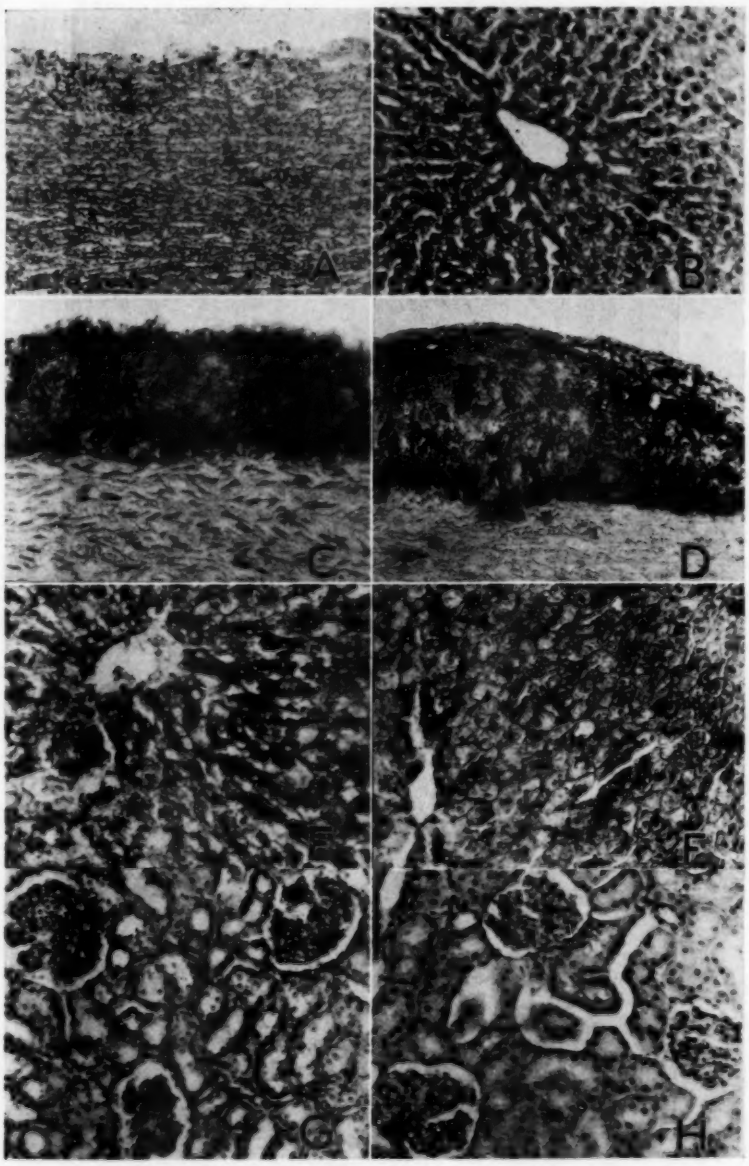

Fig. 4.—*A*, section of the aorta of an animal fed alcohol. *B*, section of the liver of an animal fed alcohol. *C*, atheromatous aortic plaque of an animal fed alcohol and cholesterol. *D*, atheromatous aortic plaque of an animal fed cholesterol. *E*, section of the liver of an animal fed alcohol and cholesterol. *F*, section of the liver of an animal fed cholesterol. *G*, section of the kidney of an animal fed alcohol and cholesterol. *H*, section of the kidney of an animal fed alcohol. The magnification of *A* and *B* is about one-half that of *C* to *H*, inclusive. All the sections were stained with sudan III.

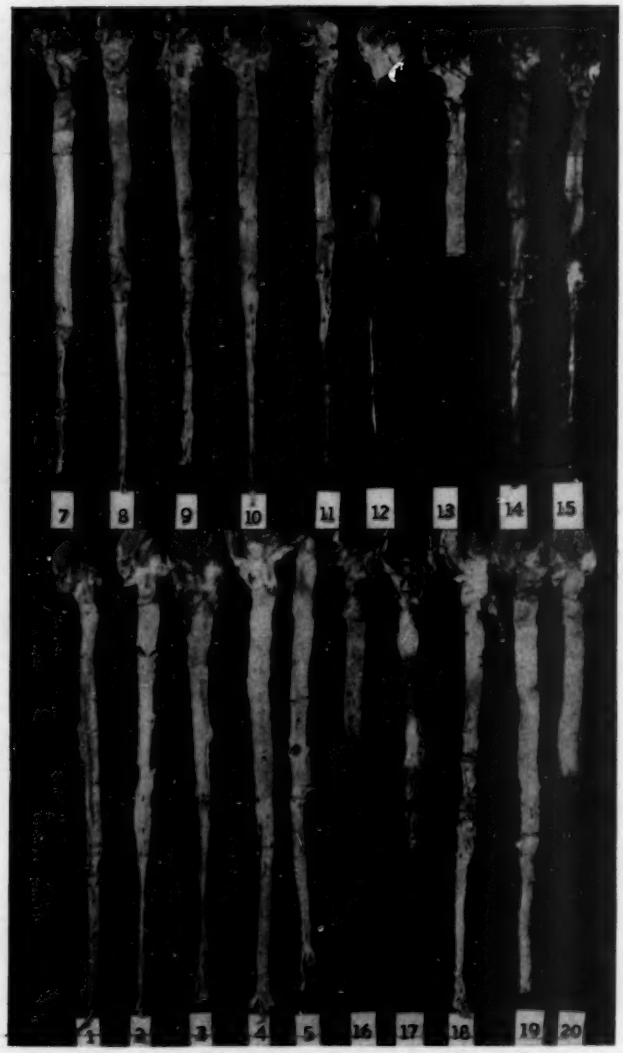

Fig. 5.—Hearts and aortas of the animals of both the groups fed cholesterol (rabbits 7 to 10 and 11 to 15), showing atherosclerotic plaques. These plaques are absent in the hearts and aortas of the group fed alcohol but no cholesterol (rabbits 1 to 5) and from the hearts and aortas of the control group (rabbits 16 to 20).

Kidneys: No differences could be seen grossly or histologically between the kidneys of the various groups of rabbits except the heavy deposits of lipoid in the glomeruli, tubules and interstitial tissue of both the group given alcohol and cholesterol and the group fed cholesterol but no alcohol. These deposits show well in figure 4 *G*. Whether or not there was some impairment of renal function which does not show histologically I cannot state definitely, though a few determinations of the blood urea about the eightieth day gave no evidence of concentration of nitrogenous substances.

Coronary Arteries and Femoral Arteries: Many atheromatous plaques were present in the coronary arteries, but none was noted in the femoral arteries. No differences due to the alcohol could be demonstrated.

SUMMARY

In a group of rabbits fed cholesterol and alcohol under the conditions described it was found that the cholesterol content of the blood rose more rapidly and to a higher level in the animals receiving both substances than in those receiving cholesterol alone but that the deposition in the tissue of the liver and aorta occurred in an inverse ratio. It was also found that a group of rabbits that received only alcohol in addition to a standard diet of grain showed a slightly higher cholesterol content of the blood and a definitely lower cholesterol content of the aorta than a control group.

SIGNIFICANCE OF TISSUE LYMPHOCYTES IN THE PROGNOSIS OF LYMPHOGRANULOMATOSIS

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The scope of this paper embraces the rôle of the lymphocytes and the lymph nodules of the lymph nodes and spleen and the filiation thereto of the prognosis in Hodgkin's disease. The other histologic alterations are mentioned only briefly, and the reader is referred to the articles by Sternberg,[1] Reed,[2] Kaufmann,[3] Ewing [4b] and finally the recent review by Wallhauser [5] for more complete information.

Sixty-three cases were studied, in thirty-nine of which biopsies had been made before the start of roentgen treatment. In twenty-nine instances, tissues were available post mortem. In fifteen of the latter instances there had been no roentgen treatment.

This investigation was undertaken originally to record the histologic changes in the lymph nodes following roentgen exposure, especially in the "various types" of lymphogranulomatosis. As the work progressed it became evident that the number of the lymphocytes and the size of the lymph nodules of the lymph nodes and the spleen decreased with the duration of the disease and the intensity of the roentgen treatment.

CHANGES IN LYMPH NODES

The cases could be divided histologically into three groups dependent on the predominance, the subordinance and the absence of lymphocytes and lymph nodules.

Considering only the biopsy specimens taken before treatment was instituted, the following classification was constructed:

1. Lymphocytes and lymph nodules fairly well preserved, at least in part, and reticulum cell proliferation only slightly pronounced (L-R type).

From the Department of Pathology, Cook County Hospital; Dr. R. H. Jaffé, director.

1. Sternberg, C.: Ergebn. d. allg. Path. u. path. Anat. **9**:360, 1903.

2. Reed, D. M.: Johns Hopkins Hosp. Rep. **10**:1933, 1902.

3. Kaufmann, E.: Pathology, Philadelphia, B. Blakiston's Son, 1929.

4. (*a*) Ewing, J.: J. A. M. A. **68**:1238, 1917; Am. J. Roentgenol. **9**:331, 1922; (*b*) Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928, p. 399.

5. Wallhauser, A.: Arch. Path. **16**:522, 1933.

2. Cellular reticulum predominant but traces of lymph nodules still discernible (R-L type).

3. Lymph nodules entirely gone, with only a few scattered lymphocytes remaining, and reticulum cell proliferation dominant (R-F and F-R types, depending on the amount of fibrosis).

The reticulum cell proliferation (R) was subdivided into: small reticulum cells resembling fibroblasts (SR); the epithelioid variety with large pale nuclei and ample cytoplasm (R); the giant reticulum cells (GR) with bizarre, huge nuclei, resembling sarcomatous cells.

For convenience and clarity, the histologic changes were indicated by numerical values. Granted that at best such a method appears awkward, it was found suitable for this purpose. The range of deviation was subdivided into five parts, with the "normal" being charted as in table 1. Thus, for lymphocytes, lymph nodules and cellular predominance, 4 to 5 was considered normal, whereas for connective tissue proliferation, giant cells (nonspecific), granulocytes, eosinophils, plasma cells and mitosis 0 to 1 was considered normal. Translated into the accepted terminology, 1 referring to the latter group means normal to slight, 2 to 3 moderate and 4 to 5 marked transformation, while, referring to the former, the reverse is true.

Lymphocyte-Reticulum Cell Type (L-R).—There is no general agreement as to the earliest changes occurring in lymphogranulomatosis. One group records the proliferation of the cellular reticulum of the secondary nodules, or germinal centers, and of the sinuses as primordial (Sternberg,[1] Reed,[2] Simmons,[6] Terplan and Mittelbach[7] and Ewing[4]), while another group states that hyperplasia of the lymphoid cells is primary (Longcope,[8] Fabian,[9] Lemon and Doyle,[10] Symmers[11]). Most authors agree that both of these changes appear in the early stages of Hodgkin's disease, but it is only with the presence of Sternberg-Reed cells that a definite diagnosis can be established.

What has been considered as "early Hodgkin's disease" in the literature does not mean necessarily that the disease is of short duration. In fifteen cases of "early Hodgkin's disease" (table 1 and fig. 1) it was observed that, contrary to current opinion, this type of lesion may be found four years after the onset of the disease and before

6. Simmons, C. C., and Benet, G.: Boston M. & S. J. **177**:819, 1917.

7. Terplan, K., and Mittelbach, M.: Virchows Arch. f. path. Anat. **271**:759, 1929.

8. Longcope, W. T.: Am. J. M. Sc. **164**:781, 1922.

9. Fabian, E.: Arch. f. klin. Chir. **91**:317, 1909.

10. Lemon, W. S., and Doyle, J. B.: Am. J. M. Sc. **162**:526, 1921.

11. Symmers, D.: Am. J. M. Sc. **167**:313, 1924; **174**:9, 1927.

roentgen treatment has begun. (In the following tables the time of the start of treatment corresponds to the time of the first biopsy.) The average duration of the disease at the time of the first biopsy was 1.37 years. Cases of "early Hodgkin's disease," therefore, may extend over a long period.

The lymphogranulomatous process was observed both in the germinal centers and in the cell cords, but with marked predominance in the former. The pleomorphic cellular reticulum appeared as if hemmed in by the surrounding lymphocytes. Newly formed lymphocytes were present only in slight amounts in the secondary nodules, while in the pulp, especially between the follicles, a more lively lymphocytic response was evident with the formation of pseudonodules. The total number of lymphocytes was in all cases less than normal, and the number of the true nodules was reduced (table 1). Giant cells of the various types as well as polymorphonuclear leukocytes, eosinophils and plasma cells were in evidence. Capsular invasion, mitosis and fibrosis were absent or slight. Generally, the normal architecture of the node was only partially preserved. A definite diagnosis was not made without the presence of the Sternberg-Reed type of giant cell.

The impression derived from the conglomerate histologic picture was that the abnormal process was hindered from immediate progression. In subsequent biopsies in two cases this defense mechanism was found destroyed, and the lymph nodules had disappeared (table 7).

Of the fifteen patients of this group, eleven are living, three are dead, and of one the status is unknown. The average duration of life since the onset of the disease for the entire group is 4.35 years; for the living, 3.93 years, and for the dead, 4.5 years. In one instance in which no treatment had been given, the disease had been present for 5 years as an incidental to a mitral stenosis, from which the patient died.

The bearing of the early induction of treatment in this group seems significant. The average time of onset of treatment for the patients still alive was 1.04 years, while for those deceased it was 3.5 years. In the latter group, however, the duration of life after roentgen treatment was 0.75 year.

Reticulum Cell-Lymphocyte Type (*R-L*).—The number of lymphocytes was greatly reduced, and the nodules were seen only in bare skeleton, appearing as narrow rims about the ever advancing granulomatous tissue. In many sections the primary nodules had entirely disappeared. One could trace the compression or encroachment on the lymph nodules until there remained only a narrow subcapsular zone of lymphocytes.

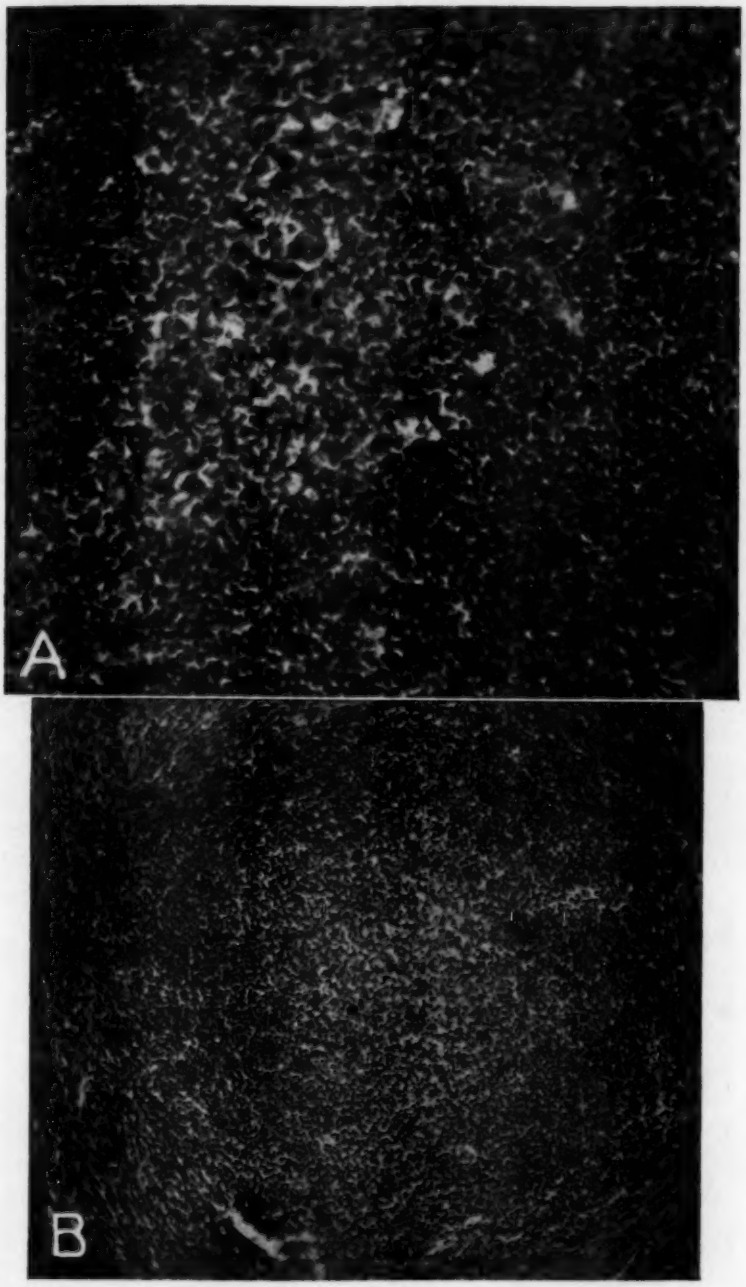

Fig. 1.—*A*, lymph node showing the L-R type of lesion. Note the lymphogranulomatous process surrounded by lymphocytes (hematoxylin and eosin; × 200). *B*, lymph node showing the L-R type of lesion. This is a more advanced stage than that shown in *A*. Note the dark staining Sternberg-Reed cell in the center of the field (hematoxylin and eosin; × 100).

TABLE 1.—*Observations in Cases of Lymphocyte-Reticulum Cell (L-R) Type*

Type	Patient Living or Dead	Duration of Life After Onset, Yr.	Time of Start of Roentgen Treatment After Onset of Disease, Yr.	Duration of Life After Start of Irradiation, Yr.	Courses of Treatment	C.T.: Cells	Reticulum Cell Proliferation	Giant Cells: Mononuclear	Giant Cells: Polynuclear	Giant Cells: Sternberg-Reed	Fibrosis	Lymphocytes	Nodules	Neutrophils	Eosinophils	Plasma Cells	Microscopic Necrosis	Capsule Invasion	Mitosis
Normal						1:4	0-1	0-1	0	0	0-1	4-5	4-5	0-1	0-1	0-1	0	0	0-1
L-R	L	5.6	1.0	4.6	3	1:4	3	1	0	1	1	4	4	1	1	1	0	0	0
L-R	L	3.5	2.0	1.5	2	2:3	3	0	0	1	2	3	2	1	2	1	0	0	0
L-R	L	1.0	0.4	0.6	2	1:4	2	0	0	?*	0	4	2	0	0	0	0	1	0
L-R	L	1.5	0.16	1.34	2	1:4	2	1	0	1	1	4	1	1	1	1	0	0	1
L-R	?	5.25	0.75	4.5	4	1:4	3	1	1	1	2	3	2	1	1	1	0	0	1
L-R	L	13.0	0.5	12.5	2	1:4	1	1	0	1	1	4	3	0	0	0	0	2	2
L-R	L	2.8	2.0	0.8	1	2:3	3	2	1	1	2	3	2	1	1	1	1	1	1
L-R	L	4.5	0.16	4.34	4	2:3	2	1	0	1	3	3	3	1	2	1	1	1	1
L-R	L	4.25	2.0	2.25	4	2:3	3	1	0	2	2	3	1	2	2	2	2	0	0
L-R	L	1.16	0.8	0.36	1	1:4	2	1	1	1	1	4	3	0	0	0	3	0	0
L-R	D	4.5	4.0	0.5	2	1:4	3	1	1	1	2	3	2	1	1	1	1	0	1
L-R	D	5.0	No treatment			1:4	2	1	1	1	1	4	3	0	1	0	0	0	0
L-SR	L	2.8	0.5	2.3	4	1:4	2	1	1	1	1	4	2	1	2	1	0	0	0
L-SR	L	3.16	2.0	1.16	1	1:4	2	1	0	1	1	4	3	1	1	1	0	0	1
L	D	4.0	3.0	1.0	3	1:4	1	0	0	0†	2	4	0	0	0	0	3	2	1

* Before roentgen exposure, lymphatic leukemia; after roentgen exposure, GE-F.

† Before roentgen exposure, lymphatic leukemia; after roentgen exposure, GE-R.

Attempts at regeneration of lymphocytes were evidenced in the presence of scattered medium-sized to large lymphocytes. There was no attempt at nodule formation. Proliferation of fibrocytes and connective tissue formation were prominent, as were giant cells, mitotic figures and capsular invasion (table 2).

The average duration of life after the onset of the disease for this group was 2.29 years; for the living, 2.11 years (eight cases), and for the dead, 2.56 years (six cases). Treatment was begun 0.94 year after the onset of the disease as an average for all cases; for the living, 1.01 years; for the dead, 0.87 year. Whether the timely start of treatment will prolong the life of these persons cannot be stated as yet (see table 2).

Reticulum Cell-Fibrosis Type (R-F or F-R).—The histologic picture as revealed by the biopsies in this group showed that the number of lymphocytes was reduced to a minimum and that the lymph nodules were absent. The giant reticulum cells, which were not prominent in the L-R type and which predominated in one case of the R-L type, appeared in four cases of this group. The latter observation corresponds to that of "Hodgkin's sarcoma" as recorded in the literature.

Whether the presence of lymphocytes in sufficient quantities prevents the development of the giant cells, or whether these cells develop so rapidly that they quickly destroy the lymphocytes, cannot here be adjudged. The "malignancy" of this type of lesion is not always greater than that of the medium or small reticulum cell types as far as the length of life is concerned. It is the presence or absence of lymphocytes and lymph nodules that is paramount in determining the prognosis.

The other important histologic alterations of this group were the prevalence of mitotic figures and capsular invasion and the frequency of necrosis. Fibrosis, granulocytes of the neutrophilic and eosinophilic order and plasma cells were as common or less so than in the R-L groups (table 3).

The average duration of life for the ten cases was 1.14 years; of the dead it was 1.18 years (nine cases) and of the living 0.75 year (one patient who was doing poorly at the time of this report). Treatment was begun early in the disease, the average time being 0.61 year after the onset, yet the duration of life after the start of irradiation was 0.45 year.

Summary of Tissue Changes in all Types.—Table 4 depicts the averages for the groups, based on the figures for the individual cases given in the first three tables. The most striking differences are those of the lymphocytes and the lymph nodules. It is recorded that type

TABLE 2.—*Observations in Cases of Reticulum-Lymphocyte (R-L) Type*

Type	Patient Living or Dead	Duration of Life After Onset, Yr.	Time of Start of Roentgen Treatment After Onset of Disease, Yr.	Duration of Life After Start of Irradiation, Yr.	Courses of Treatment	C.T.: Cells	Reticulum Cell Proliferation	Giant Cells			Fibrosis	Lymphocytes	Nodules	Neutrophils	Eosinophils	Plasma Cells	Microscopic Necrosis	Capsule Invasion	Mitosis
								Mononuclear	Polynuclear	Sternberg-Reed									
Normal						1:4	0-1	0-1	0	0	0-1	4-5	4-5	0-1	0-1	0-1	0	0	0-1
R-L	L	2.4	1.0	1.4	2	2:3	3	1	0	1	2	3	0	1	1	1	1	0	0
R-L	L	1.75	1.0	0.75	3	2:3	3	1	1	1	2	2	1	1	1	1	0	1	2
R-L	D	3.0	0.75	2.25	1	2:3	2	1	0	1	3	2	0	2	3	2	1	1	0
R-L	L	1.33	1.33	0	1	2:3	3	2	1	1	2	2	0	2	2	2	0	1	2
SR-L	D	2.16	0.08	2.08	2	2:3	3	0	0	1	2	2	0	1	2	1	0	1	..
SR-L	L	2.8	0.3	2.5	3	2:3	3	2	1	1	2	2	0	1	1	2	0	2	3
R-L-F	L	3.5	2.0	1.5	1	3:2	2	1	0	0	3	2	1	1	1	1	1	1	0
R-L-F	D	1.8	0.8	1.0	3	3:2	3	1	0	1	3	2	0	1	1	1	0	0	0
R-L-F	L	1.6	0.6	1.0	4	4:1	2	0	0	0	4	3	1	1	1	1	1	0	0
SR-L-F	L	1.75	0.8	0.95	2	4:1	2	1	0	1	4	3	1	1	2	1	0	0	1
SR-L-F	D	3.3	0.08	3.24	4	3:2	2	1	0	1	3	3	1	1	1	1	1	0	0
SR-L-F	L	1.75	1.0	0.75	3	2:3	3	1	0	1	3	2	0	0	2	1			
SR-L-F	D	2.5	1.5	1.0	1	3:2	2	2	0	1	3	2	1	0	1	0	0	1	1
GR-L	D	2.6	2.0	0.6	1	2:3	3	3	2	2	2	2	0	1	2	2	0	1	2

TABLE 3.—*Observations in Cases of Reticulum Cell-Fibrosis (R.-F) Type*

Type	Patient Living or Dead	Duration of Life After Onset, Yr.	Time of Start of Roentgen Treatment After Onset of Disease, Yr.	Duration of Life After Start of Irradiation, Yr.	Courses of Treatment	C.T.: Cells	Reticulum Cell Proliferation	Giant Cells: Mononuclear	Giant Cells: Polynuclear	Giant Cells: Sternberg-Reed	Fibrosis	Lymphocytes	Nodules	Neutrophils	Eosinophils	Plasma Cells	Microscopic Necrosis	Capsule Invasion	Mitosis
Normal						1:4	0–1	0–1	0	0	0–1	4–5	4–5	0–1	0–1	0	0	0	0–1
R–F	D	2.9	1.0	1.9	3	3:2	3	1	0	1	3	1	0	1	1	1	2	1	1
R–F	D	0.66	0.58	0.08	1	3:2	3	1	0	1	3	1	0	1	2	1	2	0	1
SR–F	D	0.75	0.4	0.35	1	3:2	3	1	0	1	3	1	0	0	0	0	3	3	..
SR–F	D	1.16	0.66	0.5	1	3:2	3	1	0	1	3	1	0	2	1	2	2	2	1
SR–F	D	1.25	0.66	0.59	2	2:3	3	1	0	1	2	1	0	1	1	1	1	0	1
GR–	D	0.75	0.59	0.08	1	1:4	4	4	0	1	0	1	0	0	1	0	0	3	3
GR–	D	0.59	0.42	0.16	1	1:4	4	4	2	1	1	1	0	0	0	0			3
GR–F	D	1.0	0.75	0.25	1	2:3	4	4	2	2	2	1	0	0	0	0	2	2	1
SR–GR	D	1.66	1.5	0.16	2	2:3	2	2	2	2	2	1	0	2	2	2	1	1	2
F–R	L*	0.75	0.25	0.5	2	3:2	3	2	1	1	3	1	0	1	1	1	3	0	0

* Patient doing poorly.

TABLE 4.—*Summary of Observations in All Cases*

Type	Duration of Life After Onset, Yr.: All	Living	Dead	Time of Start of Roentgen Treatment After Onset of Disease, Yr.: All	Living	Dead	Duration of Life After Start of Irradiation, Yr.: All	Living	Dead	Courses of Treatment	C.T.: Cells	Reticulum Cell Proliferation	Giant Cells: Mononuclear	Giant Cells: Polynuclear	Giant Cells: Sternberg-Reed	Fibrosis	Lymphocytes	Nodules	Neutrophils	Eosinophils	Plasma Cells	Microscopic Necrosis	Capsule Invasion	Mitosis
L–R	4.35	3.93	4.50	1.37	1.04	3.50	2.69	2 89	0.75	2+	1:4	2	1—	0.5—	1—	1.5	4—	2+	1—	1	1—	1—	0.5—	0.5+
R–L	2.29	2.11	2.56	1.01	0.94	0.87	1.35	1.10	1.69	2+	3:2	2.5	1+	0.5—	1	3—	2+	0.5—	1	1.5	1+	0.5—	0.5+	1—
R–F	1.14	0.75	1.18	0.68	0.25	0.62	0.45	0.50	0.45	1.5—	2:3	3+	2+	1—	1+	2.5—	1	0	1	1	1	1.5	1+	1.5
Normal											1:4	0-1	0-1	0	0	0-1	4-5	4-5	0-1	0-1	0-1	0	0	0-1

L-R has slightly below the normal amount of lymphocytes, type R-L much less and R-F only a trace. The same is true for lymph nodules; indeed, in the last group, the nodules had entirely disappeared.

Coincidently, the average duration of life after the onset of the disease seems to be directly proportional to the numbers of lymphocytes and lymph nodules. Thus the total duration of life for the three groups was 4.35, 2.29 and 1.14 years, respectively. Embracing only the cases in which the patients were dead, it was 4.5, 2.56 and 1.18 years, respectively. The variation in each group was not marked; for the cases in which the patients were deceased the duration was, L-R type, from 4 to 6 years; R-L type, from 1.8 to 3.3 years; R-F type, from 0.59 to 1.66 years (only one person lived for 2.9 years). The percentages of living patients in the three groups were 79, 43 and 10, respectively.

The appearance of giant reticulum cells, capsular invasion and mitosis seems dependent on diminution in the numbers of lymphocytes and primary nodules. It is generally believed that there is no parallelism between the histologic picture and the prognosis (Favre and Croizat [12] and Wallhauser [5]), and this is true when the lymph nodules have been abolished, but their presence seems definitely associated with a prolongation of life. Fibrosis, neutrophils, eosinophils and plasma cells have no bearing on the clinical course of the disease and were found to be the most conspicuous in the R-L group.

The timely onset of irradiation in the R-L and R-F groups does not seem to have influenced the course of the disease. In the L-R group the early institution of treatment seems to have been beneficial, although the time of starting it was 1.04 years as compared with 1.01 and with 0.68 year for the other two groups.

CHANGES IN THE SPLEEN

Lymphogranulomatosis is primarily a disease of the lymphatic tissue although no organ is spared by this condition. As the spleen is an important part of the lymphatic system, it too is expected to undergo changes similar to those found in the lymph nodes. However, cognizance of the fact must be taken that a fraction of said system may carry on the normal function, as is true with the liver and the kidney. It is not necessary, however, to deplete the entire body of lymphocytes before the host succumbs. For this reason it may be possible to find a portion of the lymphatic system still intact on postmortem examination.

12. Favre, N., and Croizat, P.: Ann. d'anat. path. **8**:838, 1931.

Macroscopically, the spleen in the majority of cases is enlarged (from 65 to 75 per cent, Ziegler [13]) and involved by the process. As this study was mainly histologic, the reader is referred to the articles by Fraenkel,[14], Kaufmann [3] and Wallhauser [5] for more details.

Microscopically, the earliest changes are found in the malpighian bodies (Ziegler [13]); the pulp may also be involved. Here, as with the

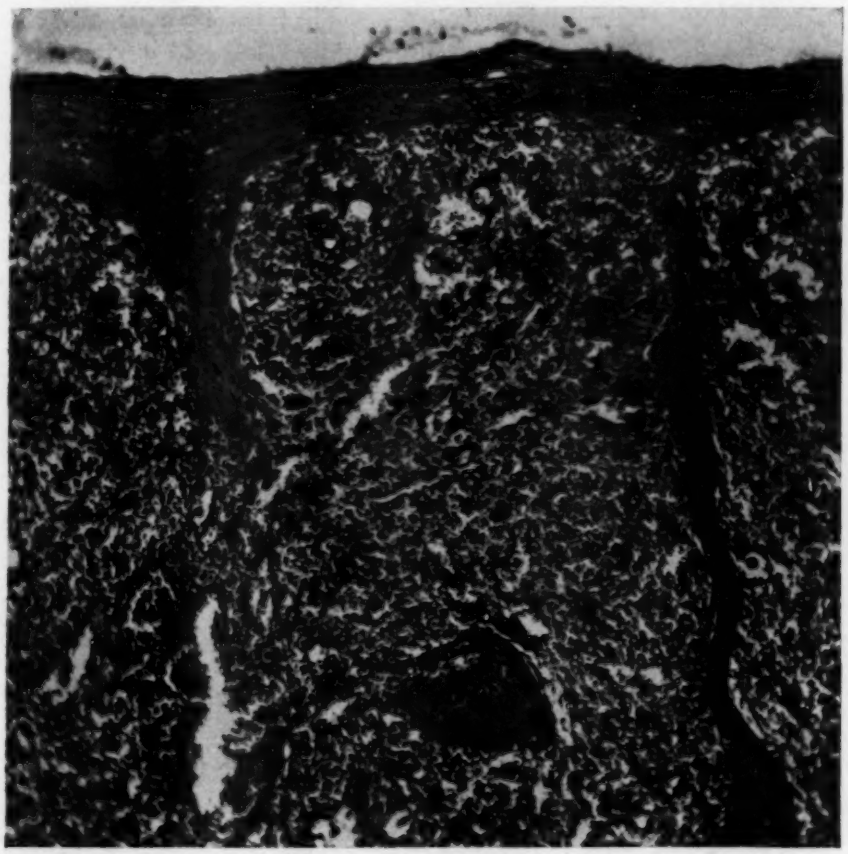

Fig. 2.—Spleen showing disappearance of lymph nodules followed by fibrosis (hematoxylin and eosin; × 100).

lymph nodes, the granulomatous process compresses or encroaches on the lymph nodules until they finally disappear or only a trace remains (fig. 2). If the pulp is first to be involved, pseudonodules form, but

13. Ziegler, K.: Die Hodgkinsche Krankheit, Jena, Gustav Fischer, 1911.

14. Fraenkel, E.: Lymphomatosis granulomatosa, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, p. 349.

these as well as the primary nodules are soon exterminated. Fibrosis, hemorrhage, necrosis, amyloid and hyaline changes are present singly or together.

As the splenic sections were obtained at autopsy, only the end-results were divulged. Yet here and there one found evidences of the various stages described and reconstructed in foregoing sections of this paper. In all, twenty-nine spleens were examined; fourteen had received roentgen treatment, while fifteen had not been exposed to roentgen rays. For convenience a procedure was adopted in recording the histologic changes similar to that used in the case of the lymph nodes (tables 5 and 6).

TABLE 5.—*Changes in Lymph Nodes and Spleens with Roentgen Treatment*

Lymph Nodes			Spleen							
Type Before Roentgen Treatment	Type During Roentgen Treatment	Type at Autopsy	Specific Changes	Lymphocytes	Nodules	Reticulum Cell Proliferation	Fibrosis	Neutrophils	Eosinophils	Necrosis
L–	..	GR-F	+	1	Trace	2	4	0	0	0
..	L-R	F-R	0	3	1	3	2	1	2	2
..	SR-L-F	F-R	0	1	1	2	3	0	0	0
SR-L-F	..	F-SR	+	1	Trace	2	3	1	1	3
..	R-F-L	R-F	+	1	Trace	3	3	2	2	3
SR-F-L	SR-F	F-R	+*							
SR-F	..	F-SR	*							
..	..	SR-F	0	1	Trace	2	2	0	0	0
..	..	SR-F	+	2	1	3	2	1	1	2
..	..	R-F	0	2	1	2	3	1	1	0
..	..	R-F	+	1	Trace	2	2	1	1	0
..	F-R	F-R	+	1	1	2	3	1	1	3
GR-F	..	GR-F	+	1	Trace	1	3	0	0	2
..	..	GR-F	+	2	Trace	2	3	2	1	
Average number involved, 8.....			1+	Trace	2+	3—	1—	1—	1.5+	
Average number not involved, 4.....			2—	1	2+	3—	0.5—	1—	0.5	
Normal.....			4-5	4-5	1-2	0-1	0-1	0-1	0	

* Sections lost.

Changes Following Roentgen Treatment.—Of the fourteen spleens, eight showed specific microscopic involvement (57 per cent). The slides examined were taken from the areas giving the most ground for suspicion, but, as serial sections were not carried out, isolated lesions, which are not uncommon, may have been overlooked in the remaining 43 per cent.

A striking aspect of this series was that irrespective of specific involvement the lymphoid tissue was greatly reduced, the reduction being less pronounced in the noninvolved organs.

The nodules, if not entirely replaced, showed central fibrosis with a small rim of lymphocytes (fig. 2). Other changes, as indicated in table 5, were not markedly different in the involved and noninvolved spleens.

Changes Without Roentgen Treatment.—The fifteen organs examined were surprisingly similar to those described in the foregoing paragraphs. Necrosis, hyalinization, hemorrhage and fibrosis were marked in this group also. Here, too, the numbers of lymphocytes and lymph nodules were markedly reduced. In the specifically affected spleens (85 per cent) these changes were more advanced than in the spared organs (table 6).

Examination of the lymph nodes of the group unveiled the paucity of lymphatic tissue. They all fell under the R-F type. The frequency of the giant reticulum (GR-F) type (27 per cent) was similar to that found post mortem in the cases in which roentgen treatment had been given, irrespective of their type before exposure to roentgen rays.

TABLE 6.—*Changes in Lymph Nodes and Spleens Without Roentgen Treatment*

		Spleens						
Lymph Nodes, Type	Specific Changes	Lymphocytes	Nodules	Reticulum Cell Proliferation	Fibrosis	Neutrophils	Eosinophils	Necrosis
SR-F	+	1	Trace	1	3	1	1	3
SR-F	0	2	2	2	2			0
SR-F	+	2	Trace	3	3	1	1	1
SR-F	+	2	Trace	2	2	1	1	3
SR-F	+	2	2	3	3	1	1	0
SR-F	+	1	Trace	2	3	1	0	1
SR-F	+	2	2	3	3	1	1	1
SR-F	+	2	2	2	3	2	2	1
R-F	+	1	1	3	3	1	1	1
R-F	+	2	1	4	2	1	1	1
GR-F	+	1	Trace	2	2	2	1	3
GR-F	+	1	1	3	3	1	1	1
GR-F	0	2	2	3	2	0	0	0
GR-F	+	2	1	2	3	1	1	2
NEC-F	+	1	Trace	1	2	1	1	4
Number involved, 13....		1.5	1	2.5	2.5	1	1	1.5
Number not involved, 2		2	2	2.5	2.0	0	0	0
Normal.....		4-5	4-5	1-2	0-1	0-1	0-1	0

Summary of Splenic Changes.—Except for the difference in the percentages of involved spleens (which may be discarded by the law of probabilities), the histologic modifications of the two groups (i. e., treated and untreated) are not dissimilar. It is of significance that here, as with the lymph nodes, the reduction of the lymphoid tissue was striking, especially in the involved organs, and this observation was true for both the specifically involved and the noninvolved organs.

The process appeared earlier in the germinal centers and extended peripherally to compress or destroy the nodule. In many instances, the nodule was found centrally fibrosed with but a few circumventing lymphocytes. More rarely, when the granuloma was seen first in the pulp, especially between the nodules, pseudonodules formed. These were soon destroyed as the condition progressed and compressed them.

EFFECT OF RADIATION ON LYMPHOGRANULOMATOUS PROCESS

The effect of radiation on lymphogranulomatous lymph nodes has not received a great deal of attention from the histologic standpoint. Fox and Farley [15] observed that there was a reduction in the number of reticulum and giant cells with an increase of fibrous connective tissue. Only few lymphocytes and lymphoblasts remained (also, Laignel-Lavastine and Coulaud [16]). Phennuegweth [17] described the disappearance of giant cells, or cells with phagocytic properties, and replacement by fibrous tissue. The node involved in Hodgkin's disease differs from the normal one in that it does not return to normal after irradiation but the abnormal process goes on to necrosis, hyalinization and fibrosis.

To compare more readily the histologic deviations of the lymph nodes before and after treatment in this series, table 7 was constructed. It will be noted that in all cases in which lymphocytes or lymph nodules were present in moderate numbers before or during treatment, the numbers were markedly reduced at the postmortem examination (tables 7 and 5). If biopsy specimens were taken during the course of treatment a gradual reduction of lymphoid tissue was seen to occur. Medium-sized and large lymphocytes were noted scattered about, indicating an attempt at regeneration. The number of giant cells usually increased or remained stationary. With fibrosis and hyalinization a reduction in their number became evident. In those instances in which an increase was manifest, bizarre-shaped mitotic figures appeared, and many of the cells assumed huge proportions. Polymorphonuclear neutrophils and eosinophils and plasma cells increased in numbers or remained stationary. Fibrosis and necrosis increased or remained stationary, rarely decreasing. Cellular reticulum proliferation decreased.

Reconstructing the entire process, one can say that when intensive roentgen therapy is used, the primary effect is directed toward the lymphocytes as well as toward the granulomatous process. There is a decrease of the reticulum cell proliferation with more or less pyknosis, karyorrhexis and necrosis, or there may be an abnormal irritation with bizarre-shaped mitotic figures and irregular giant cell formation. The lymphocytes also undergo degeneration with disappearance of the lymph nodules. Neutrophils, eosinophils and plasma cells increase in number or remain stationary.

If the patient survives for an adequate length of time, there follow on these destructive changes proliferative ones, with an increase of

15. Fox, H., and Farley, D. L.: J. Radiol. **4**:261, 1923.

16. Laignel-Lavastine and Coulaud, E.: Bull. et mém. Soc. méd. d. hôp. de Paris **46**:567, 1922.

17. Phennuegweth, H.: Frankfurt. Ztschr. f. Path. **44**:85, 1932.

fibrocytes and connective tissue and the appearance of medium-sized and large lymphocytes. Finally the entire gland may be replaced by dense hyalinized fibrous tissue.

Noteworthy are three cases, in two of which the process resembled histologically lymphatic leukemia, and in one, a plasma cell granuloma.

TABLE 7.—*Changes in Lymph Nodes Following Roentgen Treatment*

Type*	C.T.:Cells	Reticulum Cell Proliferation	Giant Cells: Mononuclear	Giant Cells: Polynuclear	Giant Cells: Sternberg-Reed	Fibrosis	Lymphocytes	Nodules	Neutrophils	Eosinophils	Plasma Cells	Microscopic Necrosis	Capsule Invasion	Mitosis
(B) L–R	1:4	2	0	0	0	0	4	0	0	0	0	0	1	0
(D) R–F	3:2	3	3	2	1	3	2	0	1	2	1	1	1	2
(D) L–E (2)	2:3	3	2	1	1	3	3	2	1	1	1	3		
(P.M.) F–R (2)	4:1	1	1	1	1	4	0	0	1	1	1	4		
(B) L–	1:4	0	1	0	0	2	4		0	0	0	3	2	0
(P.M.) GR–F	3:2	2	2	1	1	3	2	0	0	0	0	0	2	1
(D) SR–L	2:3	3	2	3	2	3	2	2	3	4	1	1	1	2
(P.M.) F–SR	4:1	2	1	1	1	4	1	0	0	1	1	2	1	0
(B) SR–L–F	3:2	2	2	0	1	3	2	1	0	1	0	0	1	1
(D) SR–F	2:3	3	3	1	0	2	1	0	1	2	3	0	2	0
(P.M.) F–SR	3:2	1	1	0	1	3	1	0	2	2	3	2	2	
(D) R–F–L	2:3	3	1	1	1	2	2	1	1	1	1	0	1	0
(P.M.) GR–F (2)	2:3	3	4	4	3	3	1	0	2	2	2	2	1	2
(B) SR–F	3:2	3	1	0	1	3	1	0	0	0	0	3	3	
(P.M.) SR–F (2)	3:2	3	1	1	1	3	1	0	1	1	1	0	0	
(B) SR–F	3:2	3	1	0	1	3	1	0	2	2	1	2	2	1
(P.M.) F–SR (2)	4:1	2	1	1	1	4	1	0	1	1	1	3		
(B) R–F	3:2	3	1	0	1	3	1	0	1	2	1	2	0	1
(P.M.) F–R	4:1	1	1	0	2	3	1	0	1	1	1	3	0	0
(B) GE–	0:5	5	4	0	1	0	1	0	0	1	0	0	3	3
(P.M.) GE–F	3:2	3	1	0	2	2	1	0	0	1	1	2	3	0
(B) F–R	3:2	2	1	1	1	3	1	0	2	1	2	0		
(P.M.) F–R	4:1	2	1	1	1	3	1	0	1	1	1	4		
Cases showing number increased		3	4	5	6	7	0	0	6	5	6	9	2	3
Cases showing number decreased		7	5	2	2	1	7	4	3	3	1	2	1	5
Cases showing number stationary		3	4	6	6	5	6		4	5	6	2	7	0

* B in parentheses signifies that the type was observed before the start of roentgen treatment; D, during roentgen treatment; P.M., post mortem. A number in parentheses is the number of biopsy specimens or of sections studied.

After exposure to roentgen rays, the classic lymphogranulomatous transformation occurred (MacMahon and Parker [18] and Jaffé [19]).

The changes portrayed have been ascribed to the influence of roentgen rays, and yet a certain amount of similar modification occurs without treatment. In patients not treated (table 6), paucity of lymphocytes

18. MacMahon, H. E., and Parker, F.: Am. J. Path. **6**:367, 1930.

19. Jaffé, R. H.: Wien. med. Wchnschr. **84**:388, 1934.

and absence of lymph nodules were the rule on postmortem examination of the lymphatic system. The spleens of the treated and the untreated patients were not greatly dissimilar as regards lymphatic elements. What part the natural progress of the disease plays and what part the roentgen rays play are not easily differentiated. Most likely the action of the latter enhances the granulomatous process.

COMMENT

Not all nodes in a given instance pass through the same stage at the same time, and several biopsies are necessary to determine the actual status of the disease. A single node may, however, indicate the trend of the process, especially with regard to the lymphocytic response. In the later stages of the disease and with the death of the patient the majority of the lymph nodes are usually affected directly or indirectly.

TABLE 8.—*Observations on Lymph Nodes from Different Parts of Body in One Case*

Specimen	Type	Lymphocytes	Nodules	Giant Cells	Reticulum Cells	Fibrosis	Neutrophils	Eosinophils	Microscopic Necrosis
1	GR-F	1	0	3	2	2	2	1	1
2	GR-F	1	0	3	2	2	2	1	1
3	GR-F	2	1	3	1	1	1	1	0
4	GR-F	2	1	3	1	1	1	1	0
5	GR-F	1	0	3	2	1	1	1	2
6	GR-F	1	0	3	3	1	1	2	2
7	R-F	1	0	2	3	1	1	2	0

Table 8 demonstrates this point. Seven lymph nodes from various parts of the body in one case of lymphogranulomatosis showed that in six of the nodes the giant reticulum cells predominated. In only two glands was there any evidence of the presence of lymph nodules.

The secondary or indirect reaction in the lymph nodes brought about by the entrance of the products of tissue necrosis into the blood stream is of utmost importance. These changes may be hastened following roentgen treatment, as described by Sapowsky,[20] but may also occur without treatment. The spleen in the case cited, as well as the spleens described in table 6, illustrates that despite absence of lymphogranulomatous lesions the lymphocytes and lymph nodules were reduced in number. Reticulum cell proliferation also occurs, as was described by Jaffé.[19] The propagation of the Paltauf-Sternberg process may be related to this indirect reaction.

The blood lymphocytes usually follow the same course as those of the lymph nodes and spleen. Most writers are agreed that late in

20. Sapowsky, P. W.: Beitr. z. path. Anat. u. z. allg. Path. **94**:1, 1934.

the disease lymphopenia, both relative and absolute, occurs (Naegeli,[21] Ziegler,[13] Bunting,[22] Terplan and Mittelbach[7] and Straube[23]). Aubertin[24] found this to be true in ninety-one of one hundred cases. Following roentgen treatment in man, and experimentally in animals, leukopenia is found, especially lymphopenia (Lavedon[25] and Minot and Spurling[26]).

The bone marrow is thought by many to play a part in lymphocyte formation. There are not sufficient studies, macroscopic and microscopic, on Hodgkin's disease to allow one to make definite statements, but those who have made careful observations declare that the bone marrow is involved in the majority of cases (Symmers,[11] Tetzner[27] and Arnell[28]).

The rôle of the lymphocyte in resistance against neoplasms or infections is a mooted point. From an experimental standpoint Murphy and his co-workers[29] (Mortom, Taylor and Mahahara) have shown that the round cell reaction to tissue grafts is purposeful. Avian embryos and adult brains were without defense against heteroplastic grafts when there was an absence of round cell response but became resistant if lymphoid tissue was added. The adult organism could be made nonresistant by destroying the major portion of the lymphatic system, and the ability to withstand the propagation of foreign tissue was regained only on lymphocytic recovery.

Ribbert[30] believed that in carcinoma in man the regressive changes were brought about by the round cell infiltration (MacCarty[31]). Ewing[4] and Alter[32] expressed the belief that the round cell reaction induced by roentgen rays and radium is probably the intermediary agency in effecting favorable results with that type of therapy.

Borst[33] and his co-worker Innes[34] took the stand that the round cell reaction is dependent on regressive changes in the tumor and does

21. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Leipzig, Georg Thieme, 1919.

22. Bunting, C. H.: Bull. Johns Hopkins Hosp. **22**:114, 1911.

23. Straube, G.: Folia haemat. **44**:125, 1931.

24. Aubertin, C.: Paris méd. **2**:30, 1927.

25. Lavedon, J.: Radiophys. et radiothérapie **2**:457, 1931.

26. Minot, G. R., and Spurling, G. R.: Am. J. M. Sc. **168**:215, 1924.

27. Tetzner, E.: Frankfurt. Ztschr. f. Path. **42**:545, 1932.

28. Arnell, S.: Acta radiol. **8**:259, 1927.

29. Murphy, J. B.: The Lymphocyte in Resistance to Tissue Grafting, Malignant Disease and Tuberculosis Infection, Monograph 21, Rockefeller Institute for Medical Research, 1926.

30. Ribbert, H.: Das Karzinom des Menschen, Bonn, F. Cohen, 1911.

31. MacCarty, W. C.: J. Cancer Research **14**:394, 1930: Surg., Gynec. & Obst. **18**:284, 1914.

32. Alter, N. H.: J. M. Research **40**:241, 1919.

33. Borst, M.: Ztschr. f. Krebsforsch. **40**:3, 1933.

34. Innes, J. R. M.: Ztschr. f. Krebsforsch. **40**:527, 1934.

not influence the prognosis. Most of this work was carried out on material obtained post mortem, so that these observations cannot be taken as final. As has been shown, in the late stages of lymphogranulomatosis and with the death of the patient the lymphocytes to a great extent disappear.

Murphy and his co-workers [29] have disclosed similar results in experimental tuberculosis. By depleting an animal of the greater portion of its lymphocytes, they obtained a marked acceleration of the disease. In animals that recovered (avirulent strains being used) the blood lymphocytes were increased in number, a finding which has been substantiated in guinea-pigs.[34a]

On the basis of experimental studies as well as in man there is evidence indicating that resistance to neoplasms and infections is augmented or decreased according to the lymphocytic response of the host. It is the application of this principle that has been attempted in regard to the cases of lymphogranulomatosis described here.

What is to be gained by accepting the lymphatic tissue as part of a defensive mechanism in Hodgkin's disease? Most of the efforts in the treatment of the disease thus far have been to reduce the size of the lymph nodes clinically. This is at first accomplished with comparative ease but soon becomes more and more difficult. Regardless of the method of treatment employed thus far, the end-result is similar, i. e., a depletion of the lymphoid elements of the body. (It is for this reason that discussion of the actual dosage of roentgen radiation employed in this series has been avoided.) If the lymphatic tissue protects the host against the progression of the lymphogranulomatous process, one should aim not at clinical reduction of the lymph glands but at stimulation of the same. Undoubtedly, there are many cases in which this is impossible, but at least one may make an attempt at preserving that which remains. The marked sensitivity of lymphoid tissue to roentgen rays and radium is well known (Regaud,[35] Albertini,[36] Jeckeln,[37] Desjardins [38] and Fox [39]). How the stimulation of the lymphatic system is to be brought about cannot be answered with certainty. Murphy,[29] from an experimental standpoint, has found that very small doses of roentgen radiation, dry heat (55 C.), olive oil or certain unsaturated fatty acids stimulate lymphatic tissue. Whether

34a. Rosenthal, S. R.: Arch Path., to be published.

35. Regaud, C.: Compt. rend. Soc. de biol. **86**:787, 1922.

36. Albertini, A.: Beitr. z. path. Anat. u. z. allg. Path. **89**:183, 1932.

37. Jeckeln, E.: Beitr. z. path. Anat. u. z. allg. Path. **94**:51, 1934.

38. Desjardins, A. V.: J. A. M. A. **99**:1231, 1932; **101**:1705, 1933.

39. Fox, H., and Farley, D. L.: Am. J. M. Sc. **166**:170, 1923.

or not these methods apply to human beings is still to be ascertained, but some such method directed toward lymphocytic stimulation would be worthy of trial, as the present method (treatment with roentgen rays and radium) does not prolong the life of the patient appreciably (Wallhauser [5]).

CRITICISM

There are many who believe that in true lymphogranulomatosis the lymph nodules are always absent. Thus the description of the tissue changes as of the L-R type will be contested (fig. 1). Also, the difficulties in differentiating such conditions as simple hyperplasia, chronic hyperplastic tuberculosis (Karsner [40]), thymoma in certain instances (Ewing [4b]), the process in the nodes in glandular fever (Longcope [8]), hyperplastic chronic lymphadenitis (Terplan and Mittelbach [7]), giant follicular hyperplasia (Brill, Baehr and Rosenthal [41]), peculiar forms of reticulosis, lymphatic leukemia and simple reticulum cell hyperplasia (Jaffé [19] and MacMahon and Parker [18]) are apparent to one.

In two instances already mentioned the lymphogranulomatosis resembled lymphatic leukemia (similar cases have been described by Jaffé and by MacMahon and Parker), and in a third instance, a plasma cell granuloma. Suspicious reticulum cell proliferation was noted, but a diagnosis was reserved until subsequent biopsies revealed the true nature of the disease. (The three patients had had roentgen treatment.)

In three cases of the L-R type in which several biopsy specimens were available during the course of exposure to roentgen rays and sections obtained post mortem, the gradual progression of the lymphogranulomatous process at the expense of the lymphoid tissue could be traced.

A diagnosis of Hodgkin's disease was made only in the presence of pleomorphic reticulum cells including the Sternberg-Reed type.

SUMMARY

The duration of life after the onset of Hodgkin's disease was found to be proportional to the predominance (L-R type), subordination (R-L type) and absence (R-F type) of lymphocytes and lymph nodules in the lymph nodes. The average duration of life for groups of patients showing the three types was 4.35, 2.29 and 1.14 years, respectively.

The effect of roentgen treatment on involved lymph nodes is thought to be: (1) a decrease in the number of reticulum cells with pyknosis,

40. Karsner, H. T.: Arch. Int. Med. **6**:175, 1910.

41. Brill, F. E.; Baehr, G., and Rosenthal, N.: Tr. A. Am. Physicians **39**:371, 1924.

karyorrhexis and necrosis or an abnormal reaction of such cells with bizarre-shaped mitotic figures and irregular giant cell formation; (2) a decrease in the number of lymphocytes and lymph nodules; (3) a proliferation of fibrocytes with dense connective tissue formation and hyalinization if the original destructive phenomena are overcome.

A marked decrease in the number of lymphocytes and lymph nodules was evident in the spleen and lymph nodes in all cases (with or without roentgen treatment) on postmortem examination. This was true whether the organ was involved by the process or not and was explained by the "indirect action" of the products of destruction of tissue entering the blood stream.

It is suggested that some method devised to stimulate the lymphoid elements of the body might aid in staving off the progression of the disease.

Case Reports

"HEALED" DISSECTING ANEURYSM OF THE AORTA

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In view of the fact that only about eighty cases of "healed" dissecting aneurysm of the aorta have been recorded in the literature, it seems worth while to report an additional case.

History.—A Negress, 52 years of age, was first admitted to the Roper Hospital (service of Dr. J. H. Cannon) on March 17, 1933, because of severe pain in the epigastrium, which had begun suddenly eight hours before. The pain had been constant and had radiated through to the back, some pain being felt in the right hip and in the entire right leg. A few hours after the onset of the pain the patient began to vomit, and she continued to do so until a short time before admission to the hospital. She had been "spitting up" blood since the onset of the pain and had noted some blood in the urine. Prior to this illness she had been free from abdominal disorders, except for occasional constipation. Her previous history had been essentially unimportant, according to the hospital record. The records of the Shirras Dispensary showed that the blood pressure was 220 systolic and 130 diastolic in June 1927, and that she had hypertension near that level at each of six visits before her first admission to the hospital.

In April 1930, almost immediately after drinking two glasses of ice water, she suddenly became unable to talk, and a cough productive of blood-streaked sputum developed and lasted for a few days. Hoarseness and difficulty in swallowing followed this attack, which was apparently painless, and remained for the rest of her life. The following month she was given a complete examination of the nose and throat by Dr. R. M. Hope, but nothing was found to explain the hoarseness. In January 1931 severe pain suddenly developed in both shoulders and two days later appeared in the lumbar region. It continued in the shoulders, spreading to both wrists and hands. The pain was constant and was exaggerated by motion. Although severe, it did not impress the patient as being serious enough to require hospitalization. Fluoroscopic examination of the chest in August 1930 (Dr. R. B. Taft) was reported as follows: "There is a generalized enlargement of the aorta to about double its normal size." In 1929 and 1931 the Kolmer and Kline tests of the blood gave negative results.

Physical Examination.—The patient was in acute distress. The rectal temperature was 97 F., the pulse rate 90, the respiratory rate 24 per minute and the blood pressure 190 systolic and 110 diastolic. The pupils were small and equal and reacted well to light. The apex beat was in the fifth interspace, 2.5 cm. to the left of the midclavicular line. The heart tones were normal, and no murmurs were heard. The arteries were markedly sclerotic. There was marked tenderness in the epigastric region and in the entire lumbar region, with moderate tenderness in the suprapubic area. No abdominal distention or involuntary rigidity was present.

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There was considerable tenderness over the right tibia and femur, but no abnormality could be found in these parts except numbness and weakness. No changes were noted in the reflexes. The voice was hoarse. Laryngoscopic examination a few days after admission (Dr. Hope) showed partial paralysis of the right vocal cord, with imperfect approximation. The eyegrounds (Dr. J. F. Townsend) showed a uniformly exaggerated reflex stripe, with a diminished arterial caliber and with arteriovenous compression.

Laboratory Examination.—A few hours after the patient's admission to the hospital the urea nitrogen content was 21 mg. per hundred cubic centimeters of blood, and it rose gradually to a peak of 121 mg. four days after admission, after which it gradually fell to 13 mg. at the time of her discharge. Daily examination of the urine showed blood both grossly and microscopically for five days after admission. Hyaline and granular casts were frequent; the specific gravity varied about 1.013. On the night of admission the leukocytes numbered 28,100, with 79 per cent polymorphonuclears. The hemoglobin value was 85 per cent (Dare). The leukocyte count gradually fell to 7,600, with 65 per cent polymorphonuclears on the day of discharge, the hemoglobin then being 50 per cent (Dare). The Kolmer and Kline tests of the blood were negative. The temperature rose to 101.4 F. on the day after admission, falling gradually to normal after one week. The pulse rate varied below 130, being highest on the second day after admission and gradually tending to fall to normal. During the patient's stay in the hospital the blood pressure varied between 195 systolic and 130 diastolic and 155 systolic and 95 diastolic, showing no definite tendency toward a continuous rise or fall. At no time was there any material difference between the blood pressure readings obtained from the two arms.

Cystoscopic examination by Dr. R. B. Gantt on the day after admission showed several small ecchymotic spots in the mucosa of the bladder and evidence of pressure in the posterior wall, which was "apparently due to a pelvic mass." Specimens of urine from both ureters were bloody. Retrograde pyelography showed that the left kidney was small, but there was no other abnormality. A pelvic examination a few days later revealed no abnormality.

A roentgenogram of the chest made two weeks after admission showed "an unusually large aneurysm of the entire aortic arch," without noteworthy cardiac enlargement (Dr. Hillyer Rudisill Jr.).

Course.—During the patient's first few days in the hospital morphine was required repeatedly for the relief of pain, but after that the patient's condition gradually improved, and at the time of discharge her only complaint was of pain, weakness and numbness in the right foot and leg. On discharge the second aortic sound was noted to have a "bell-like quality." The clinical diagnosis was hypertensive cardiovascular disease, generalized arteriosclerosis, saccular aneurysm of the aortic arch and infarction of the kidney. She was discharged as improved on April 28, forty-two days after admission.

The patient was seen again in the outpatient department in May 1934, when she was complaining of dyspnea, pain in the left side of the neck, hoarseness and weakness. The blood pressure was 200 systolic and 150 diastolic. A loud harsh systolic murmur was heard at the apex but was not transmitted. The patient was treated for cardiac decompensation.

Second Physical Examination.—On June 25, 1934, the patient was readmitted to the hospital (service of Dr. Robert Wilson Jr.) because of shortness of breath and difficulty in swallowing, talking and breathing. The voice had a brassy quality. Examination showed an acutely ill Negress, who still said that her age was 52

years. The temperature was normal on admission, the pulse rate 110, the respiratory rate 28 per minute and the blood pressure 192 systolic and 140 diastolic. There was marked pulsation of the vessels of the neck, with a questionable pulsation in the episternal notch. Coarse moist râles were heard in the base of each lung, more marked on the left anteriorly. Dulness and diminished breath sounds were noted over the left side of the chest posteriorly. The mediastinum was apparently widened to the right, as determined by percussion. The heart was markedly enlarged to the left; the sounds were of fair quality. A loud, high-pitched systolic murmur was heard, which was of maximum intensity at the aortic area and at the apex. There was a tambor-like second aortic sound. Examination of the abdomen revealed no abnormality. Moderate edema of both feet was noted, especially of the left foot. A roentgenogram of the chest, taken the day after admission, showed little if any change in the appearance of the "aortic aneurysm," although the heart had enlarged greatly in the interim, the diameter now being more than 7 inches (17.8 cm.), as compared with 5 inches (12.7 cm.) in April 1933. Mottling of both lungs also was noted and was interpreted as due to pulmonary congestion.

Laboratory Examination.—The urine contained albumin (3 plus) but no casts and had a specific gravity of 1.023. The hemoglobin content was 62 per cent (Dare), and the leukocytes numbered 13,200, with 78 per cent polymorphonuclears, 20 per cent lymphocytes and 2 per cent transitional cells. The Kolmer and Kline tests of the blood were negative. The pulse rate was very irregular, ranging from 100 to 130. The temperature was irregularly elevated to 102 F. There was little improvement in the cardiac decompensation. The respiraton became more rapid, and the pulse was of poor quality.

Course.—On July 1 the patient showed marked respiratory distress, became semicomatose and died. The clinical diagnosis was syphilitic aneurysm of the aorta and bronchopneumonia.

Postmortem Examination.—Autopsy was made twenty-four hours after death. Nephrosclerosis was marked, and a large old infarct was noted in the left kidney. Cardiac hypertrophy was moderately advanced, and there were evidences of congestive heart failure. The coronary arteries were patent and appeared normal. Lobular pneumonia was present.

The most striking observation at autopsy was concerned with the aorta. Viewed from the exterior, the aorta showed a rather uniform dilatation, beginning in the distal portion of the arch and extending to the termination of the aorta. The adventitia appeared normal. When the aorta was opened the true channel was seen to be surrounded almost completely by a crescentic channel which also contained blood and which seemed to serve as a second functioning aorta. Proximally this false vessel extended into the descending portion of the aortic arch, while distally it extended well into both iliac arteries. Unfortunately the true nature of the condition was not appreciated at the time of the autopsy, and the iliac arteries were severed at a point about 6 cm. from the bifurcation. Hence no description of the complete downward extent of the dissection can be given. The false vessel continued for the length of both vessels that was preserved for examination. There was no dissection of the large vessels arising from the arch of the aorta, the dissection stopping just short of the mouth of the left subclavian artery. These two channels communicated at a point in the first portion of the descending aorta, just beyond and opposite the opening for the left subclavian artery, at the site of the termination of the ligamentum arteriosum. The orifice connecting the two was slightly gaping and slitlike, 1.5 cm. in length. Its direction was almost

transverse, but its posterior end was directed slightly downward. Proximal to the dissection the walls of the aorta were elastic and of normal thickness, the only abnormality being a few small fatty deposits in the intima, without calcification or ulceration. There was no abnormal wrinkling of the intima or thickening of the adventitia. At the orifice the intima appeared the same as elsewhere, there being no evident relationship between the location and direction of the orifice and any lesion of the intima. Likewise, the intima of the true channel along the course of the dissection showed no especial lesion, only occasional fatty deposits. No other communications between the two channels were observed.

The wall of the true and that of the false vessel were of about the same thickness, but the inner lining of the false channel was irregularly roughened and wrinkled. The wrinkles ran in various directions, some longitudinally, others transversely or obliquely. Generally, the lining was a paler white than that of the true vessel, although a few small fatty deposits were noted also in the false channel. The false vessel surrounded the true one except on the posterior surface,

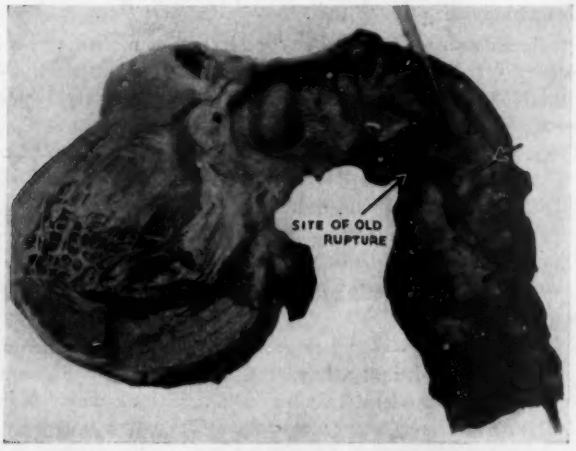

Fig. 1.—"Healed" dissecting aneurysm of the aorta, showing the communication between the two channels. The dissected channel is open; the true channel, indicated by the arrow at the right, has been sewed shut.

where the two walls merged. Traversing the lateral angles of the false channel at regular intervals were slender strands of tissue; on section some appeared to be definitely vascular channels while others were obliterated. At several points it was evident that these intercostal vessels had been torn across, as indicated by scars in the true vessel walls, while at the same levels small vessels were seen arising from the false channel to continue the vascular supply.

In the abdominal aorta such changes were even more marked. Transverse sections of both renal arteries and of the pancreatic and superior mesenteric arteries showed the true channels of these vessels to be continuous with the true aortic channel, each one completely surrounded by a secondary vessel which was continuous with the false aortic vessel. The intimal lining of the false aortic channel was well formed and appeared old. However, there were numerous thrombi rather loosely attached to the walls of this false vessel, and they had the appearance of being much more recent than the channel itself. They were dark

red and friable and showed no attempt at organization except where attached to the vessel wall. All these thrombi were about the mouths of large vessels and extended for various distances into these vessels.

It seemed not unlikely from the appearance of these thrombi that the original aortic dissection occurred some time before, was followed later either by secondary dissections along the large branches or else, in case the smaller dissections occurred at the same time as the aortic dissection, by thrombosis of these smaller channels.

Fig. 2.—"Healed" dissecting aneurysm of the aorta, showing the abdominal aorta and the first part of the iliac arteries. The new channel is open; the true channel is not.

At any rate, many of these vessels (renal, pancreatic and mesenteric arteries) were so narrowed that their tortuous lumens were probed only with great difficulty. Either of these explanations would fit with the clinical story of recurring symptoms, all probably related to the aorta. Certainly there was no definite or even suggestive evidence of secondary dissection of the aorta proper.

Microscopic sections taken from the aorta at some little distance from the dissection showed numerous scars and "faults" in the media, interrupting normal

circumferential fibers and tending to spread out somewhat between the layers of the media. At these faults the muscle was degenerated, and the elastic tissue had completely disappeared, leaving in its place rather loose hyaline fibrous tissue, in which a number of cells, apparently of endothelial origin, were noted. There was a tendency for these cells to line small slits in the media there. This tissue appeared to be fragile and bore no resemblance to the normal, regular architecture of the media.

Sections taken through the area of dissection, and including the angle where the normal aortic wall split to enclose the false channel, showed that the dissection

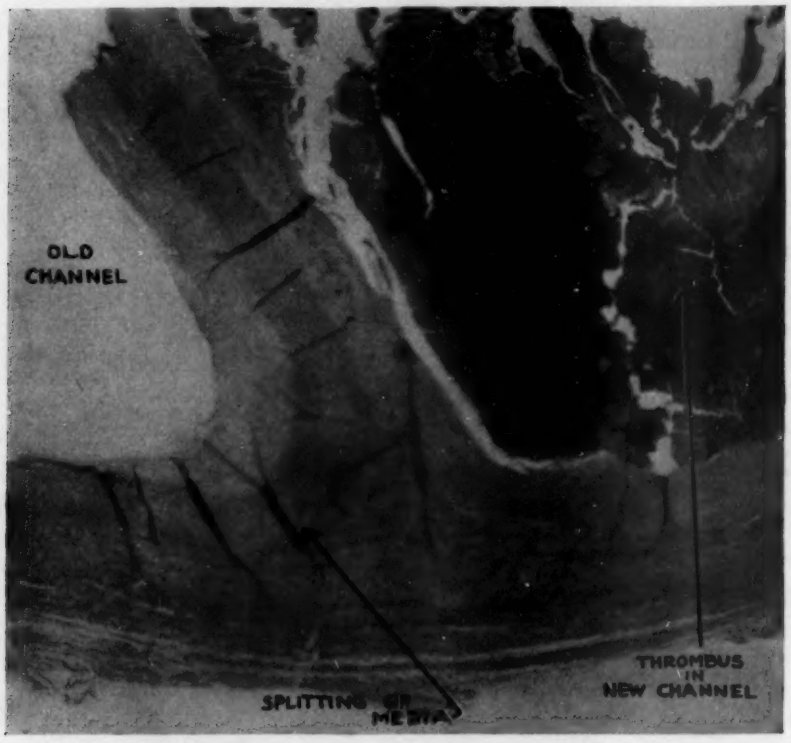

Fig. 3.—Photomicrograph showing splitting of the media to enclose the new channel. Magnification, × 12.

had occurred between the middle and the outer third of the media. The media, as included in both the wall of the old channel and the wall of the new one, was extensively degenerated, and the elastic tissue was scarce and frequently fragmented. However, the muscle and elastica were observed to maintain their normal parallel arrangement where both could be made out. In the common wall of the two channels most of the elastica was retained. While the thickness of the wall of the false channel was practically the same as that of the original vessel, the relative thickness of the component elements was greatly altered. The wall of the new channel showed a very thick and dense intima, a thin and apparently inefficient media and a greatly thickened adventitia. A section taken through one of the

adherent thrombi showed that organization was beginning from the base, but fibrin, shadow red cells and leukocytes could still be made out toward the surface, indicating that the thrombus was certainly not as old as the vessel itself.

Sections taken through the dissected superior mesenteric artery showed that the false vessel there was the larger of the two, and the true vessel was pushed aside and had assumed a crescentic shape, while the false vessel appeared nearly rounded, although its lumen was largely occluded by a thrombus. The intima of this vessel appeared as well developed as that of the false aortic vessel, suggesting that dissection of the two occurred at the same time. The thrombus, however, was recent and even gave the definite impression of being laminated, as from successive depositions of thrombi, with organization. Certainly the innermost thrombus was much more recent than the deeper ones. The media surrounding this false channel contained very little muscle and elastic tissue—in fact, even a small amount was found with difficulty—while the media of the true vessel was fairly well developed, although the split in the walls was evident.

In no section was there any suggestion of syphilitic disease. As a matter of fact, atherosclerosis was at a minimum, as there seemed to be little atheromatous change in the intima, even where medial defects were prominent.

COMMENT

Two points are of considerable interest in this case, in addition to the fact that another patient with an extensive dissecting aneurysm of the aorta survived the period of actual dissection.

One of these is the fact that the diffuse enlargement of the aorta was detected on fluoroscopic examination *before* the time at which the actual dissection is thought to have occurred. In 1930 the aorta was reported to be "about double its normal size." According to the record, it appears that dissection occurred in March 1933, although it must be conceded that it could have occurred in January 1931. This fact, coupled with the microscopic evidence of small slitlike spaces in the aortic media at some distance from the dissection, brings to mind the idea postulated by Babes and Mironescu [1] in 1910. These workers maintained that medial splits occurred as a result of dilatation of the aorta and of an associated medial degeneration. They said that they believed that the slits antedated the hemorrhage and that the intima ruptured, usually as a result of trauma, with an outpouring of blood into the space already prepared. Whitman and Stein [2] have reported the case of a 76 year old woman. An autopsy was performed after the body had been embalmed by the gravity method. The aorta showed a slit in the medial coat, extending from the base of the heart to within 10 cm. of the bifurcation, with the space containing "lymph" but no blood.

The other point of interest is the rapid onset of cardiac hypertrophy and decompensation following the dissection of the walls. The patient was known to have had a blood pressure of 220 systolic and 130 diastolic in June 1927 and to have had a blood pressure near that level on six subsequent occasions before her admission to the hospital in 1933. In spite of this continued hypertension for six years no mate-

1. Babes, V., and Mironescu, T.: Beitr. z. path. Anat. u. z. allg. Path. **48**:221, 1910.

2. Whitman, R. G., and Stein, H. B.: J. M. Research **44**:579, 1924.

rial cardiac hypertrophy was demonstrable roentgenographically, and there were no symptoms of decompensation. Fifteen months later the heart was markedly enlarged, and symptoms of decompensation had been present for at least a month. This cannot be explained on the basis of narrowing of the vascular bed, as that should have produced further elevation of the blood pressure, which did not occur. It appears more likely that the loss of the propulsive power of the aorta, due to the loss of the elasticity of its walls, had required a rapid enlargement of the heart to prevent stasis of blood in the arterial system.

SUMMARY

A case of dissecting aneurysm of the aorta, with recovery from the actual dissection and survival for fifteen and one-half months, is recorded.

It was noted that cardiac hypertrophy increased very rapidly after the dissection, although hypertension had already been present for at least six years, and that symptoms of decompensation were not apparent until after dissection had occurred. This is thought to be a result of the loss of elasticity of the aorta by virtue of the presence of the new channel, thus throwing the whole burden of propulsion of the blood on the heart alone.

BILATERAL GLIOMAS OF THE BASAL GANGLIONS

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Multiple tumors of glial origin have been reported by a number of authors. They are not common. Hosoi,[1] in 1930, reviewed the literature and reported a case of his own. Funk[2] reported 1 case, and Taylor[3] reported 2 cases, in 1 of which the growth was definitely multicentric. Alpers and Watts,[4] in their report on glioma of the mesencephalon, noted extension across the midline in 4 of their 10 cases. Glioblastoma multiforme is known to extend across the midline, not infrequently resulting in bilateral involvement.

We have encountered 2 cases of bilateral glioma in the region of the basal ganglions in which there were no demonstrable connections. Both these cases were in young children. In one the predominating cell type was the polar spongioblast, and in the other, the astrocyte. These 2 cases seemed of sufficient interest to us to merit a report.

REPORT OF CASES

Case 1.—A white boy, 5 years of age, was admitted to the service of Dr. A. D'Errico at the Baylor University Hospital. The chief complaints were: headache of five months' duration, contracture of the right arm for five months, contracture of the right leg for three months and incontinence of urine for three weeks.

The child apparently had been well until five months previous to admission, at which time headache was noticed and a severe cold developed. After a few days the parents noticed a diminution of ability in the use of the right arm. A jerking movement was observed on attempts to use this part. About three months previous to admission a slight limp in the right leg was observed. The child was able to walk, however, until three weeks prior to admission. From that time the leg became spastic and weak to a degree that prevented walking. At this time incontinence of urine developed also. The family history was essentially without significance.

Physical examination showed a well developed and well nourished boy. The findings were normal, except the following ones: The neck was slightly rigid. Marked, almost complete flexion of the right arm, with palmar extension of the hand, was present. The right arm was spastic, with hyperactive deep reflexes. The right leg was flexed at the knee and was slightly spastic.

The spinal fluid was normal. A ventriculogram revealed hydrocephalus on the right side, apparently secondary to a tumor of the midbrain. The ventricular fluid was clear. Changes at the suture lines, seen in the roentgenograms, suggested the presence of intracranial pressure.

From the Department of Pathology, Baylor University College of Medicine.

1. Hosoi, K.: Arch. Neurol. & Psychiat. **24**:311, 1930.

2. Funk, W. H.: U. S. Nav. M. Bull. **28**:114, 1930.

3. Taylor, F. W.: Arch. Path. **18**:347, 1934.

4. Alpers, B. J., and Watts, J. W.: Arch. Neurol. & Psychiat. **34**:1250, 1935.

The patient was in the hospital for ten days. Following ventriculography the temperature was 104 F.; the pulse rate was 178, and the respiratory rate was 38. Numerous convulsions occurred. A second ventricular puncture was followed by slight improvement for a brief period, and then the condition became critical. Death occurred on the tenth day.

Necropsy.—Postmortem examination was limited to examination of the head. The body was that of a well nourished and well developed white boy. A recent surgical wound (measuring 3 cm.) was present in the scalp, posterior to and above the level of the right ear. A trephine opening (measuring 6 mm.) in the skull was seen immediately underlying this wound. The suture lines between the frontal and the parietal bone were widened. The cerebrospinal fluid was increased but clear. The convolutions of both cerebral hemispheres were flattened, and the consistency of the cortex was uniform on both sides. The vessels of the meninges were somewhat prominent throughout. The left hemisphere was seen to be larger than the right. Examination of the base of the brain revealed no gross abnormality on the surface.

Sectioned surfaces of the brain, after fixation, revealed atrophy of the cerebral hemispheres. The cortex and white matter on the left side together measured 2 cm. in thickness, and the cortex and white matter on the right side, 2.5 cm., in the parietal areas. Section of the brain through the region of the tuber showed the region of each basal ganglion to be enlarged and the markings largely obliterated (fig. 1). Both sides presented a fairly sharply circumscribed, pale grayish-pink, firm mass. That on the left measured 5.5 cm. in the horizontal plane and 6.2 cm. in the vertical plane, and that on the right, 3.3 cm. in the horizontal plane and 3.6 cm. in the vertical plane. These nodules were uniformly firm and were free from cystic or necrotic areas. The tumor on the right side involved the region of the thalamic nuclei, with less evidence of compression of the surrounding structure than on the left. The internal capsule was apparently incorporated in the tumor on both sides. The same area was involved on the left side as on the right, but on the left side the tumor was much larger and bulged chiefly upward and into the ventricle. The tumor was apparently limited on the lateral surface by the external capsule, which was displaced laterally. The claustrum was flattened but could be identified. It apparently was not involved. The medial and inferior surfaces were sharply limited. No definite connection between the two masses could be seen on direct observation.

Histopathologic Observations.—Sections from both tumors, stained with hematoxylin and eosin, revealed a rather cellular lesion with a moderate amount of intercellular material (fig. 2). The outlines of the cells were indistinct. The nuclei were variable in size and shape, and the majority were oval or slightly elongated. The cells showed a tendency to be grouped in irregular rows and partial ring formation. The chromatin was moderately rich and was scattered throughout in granular form. Nucleoli were not prominent except in a few scattered small round nuclei, in which they were easily distinguished. For the most part, oval nuclei predominated, but in some areas round nuclei that stained more deeply could be seen in fairly large numbers. In the peripheral portions of the tumors most of the nuclei were of the rounded variety. Mitotic figures were present in moderate numbers in some areas but were rare in other fields. At the periphery of the masses evidence of compression was seen. The cells were decreased in number, especially those with elongated nuclei. In the surrounding tissue the nuclei of nearly all the small or medium-sized cells were of the round variety. The white substance throughout was richly supplied with such cells. The

ganglion cells of the cortex appeared to have the normal arrangement. Degenerative changes were noted in some, with an accumulation of glia cells about them. The ependymal lining and a narrow zone underlying it were not involved by the tumor tissue.

Sections stained with Cajal's gold chloride revealed many cells with elongated processes, such as are seen associated with polar spongioblasts (fig. 2). A few cells with thick, short vascular processes and small short processes and still others with longer multiple processes were seen. The astrocytic types of cells were seen with increasing frequency in the peripheral portions.

Fig. 1 (case 1).—Photograph of bilateral tumors of the basal ganglions.

In cresyl violet-stained sections an occasional degenerated and fragmented ganglion cell was observed. Staining of myelin sheaths revealed a few myelinated fibers in some portions of the tumor. They showed irregular staining and nodular thickening. The fiber tracts at the periphery showed some distortion by compression, and nearest the tumor tissue the myelin sheaths stained irregularly.

Multiple sections taken through the floor of the third ventricle revealed no direct extension of the tumor from one side to the other. Blocks were cut about 5 mm. in thickness, and several sections were taken from each block. Sections from the pons were studied for evidence of extension of the tumor process into this part, but none was found.

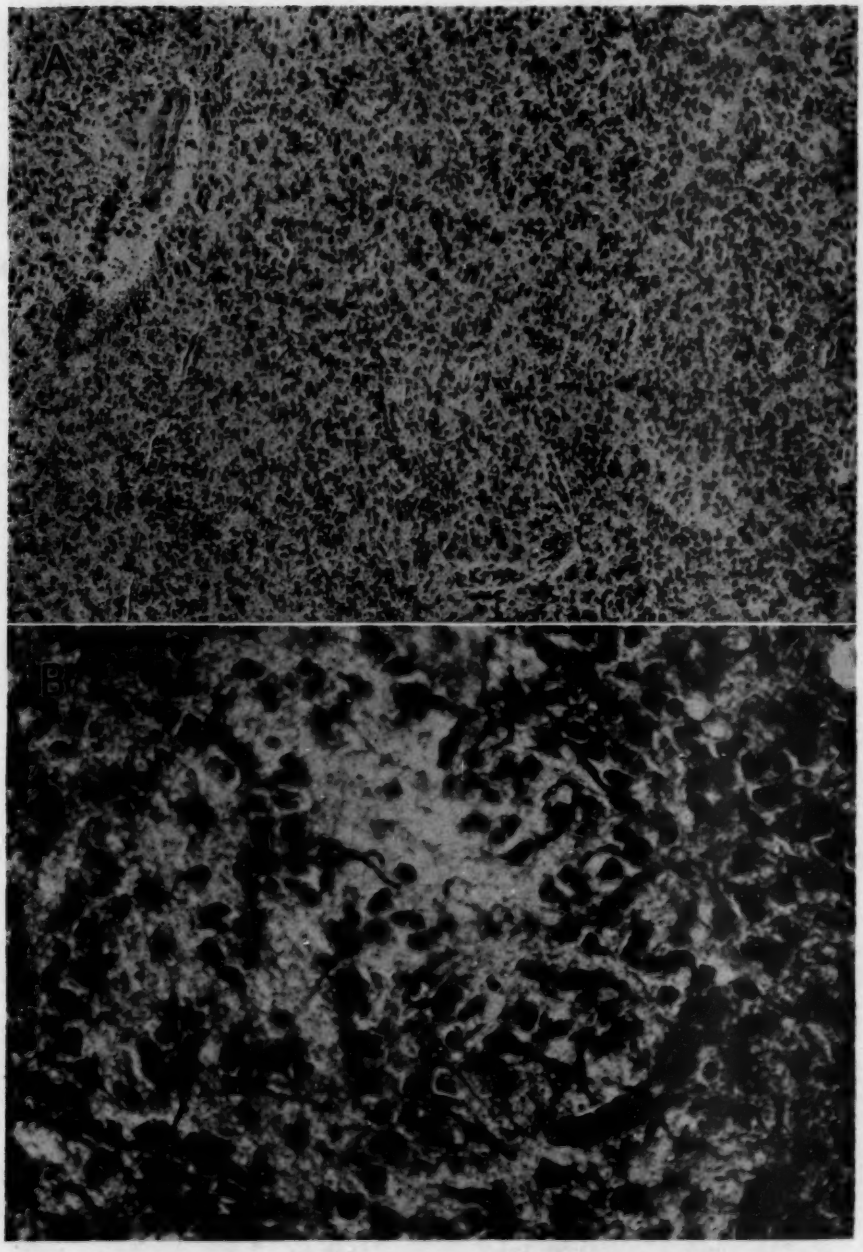

Fig. 2 (case 1).—*A*, photomicrograph of section of tumor, stained with hematoxylin and eosin, showing moderate cellularity and a tendency of the cells to be grouped in imperfect rings and palisades. *B*, section stained with gold chloride, showing many cells possessing long processes resembling spongioblasts.

CASE 2.—A white girl, aged 34 months, who was admitted to the Bradford Memorial Hospital for Babies, had apparently been well until she was 11 months of age. At that time she began having "peculiar spells" simulating convulsions. It was thought they were probably due to an injury at birth. Such spells occurred irregularly on an average of once a week and came on without warning. The child cried and became spastic, apparently without loss of consciousness. Feeding had been a difficult problem, and from the age of 11 months she was fed with a tube about "half the time." Attacks of vomiting also occurred, and at times there was diarrhea. These attacks continued and became more severe up to admission. An internal squint of the left eye was noted. The pulse rate averaged 116 and the temperature 97.8 F., and the spinal fluid was normal. The urine contained a few pus cells and albumin (2 plus) on several examinations. The red blood cells numbered 5,060,000; the white blood cells, 8,100.

Necropsy.—The body was that of a well developed white girl (slightly undernourished) about 4 years of age. The pupils were regular and equal and measured 7 mm. in diameter. The subcutaneous fat measured 4 mm. in thickness. A small amount of fibrinous exudate was present on the left pleural surface. The thymus was not large. The left lung crepitated throughout, but feebly in the posterior portion. Several dark red areas were present in the upper lobe of the left lung. The upper lobe of the right lung crepitated feebly, and the apical portion was dark red, with a firm grayish-red cut surface. The middle lobe was nearly entirely consolidated. The lower lobe of the right lung was heavy, grayish red and noncrepitant. The heart was dilated to a moderate degree. The remainder of the viscera revealed gross changes indicative of parenchymatous degeneration. Anomalies were not observed.

The scalp revealed no noteworthy gross changes. The dura was unchanged. A larger amount of subarachnoid fluid was present on the left side than on the right. The meninges were transparent. The posterior portion of the left sylvian fissure was slightly more prominent than that of the right, and the consistency of the two hemispheres was about the same. The lateral ventricles contained a small amount of blood-tinged fluid, and they were not noticeably enlarged. The basal ganglions were seen as prominent masses bulging into the ventricles. They were both very firm in consistency. The surfaces were slightly irregular, but the linings of the ventricles were everywhere smooth. Multiple sections through these regions revealed enlargement of the basal ganglions to an equal degree on the two sides. The more central portions were very firm and were pale whitish gray. The firm masses measured 2 by 3 cm. and 2.2 by 3.3 cm., respectively. The internal capsule was indistinct. In some regions a definite demarcation was not seen, the lesion appearing to fade out into the surrounding tissue and becoming less firm and grayish pink. The floor of the third ventricle was not changed. Multiple sections of the cerebral hemispheres, cerebellum, pons, medulla and upper part of the spinal cord revealed no discernible change on gross inspection.

Histopathologic Observations.—Sections from both tumors, stained with hematoxylin and eosin, revealed moderately cellular masses in which the outlines of the cells were indistinct (fig. 3). The nuclei varied in size to a moderate degree, and occasional binucleated cells were observed. The majority of the nuclei were rounded. The chromatin was regular and uniformly distributed. The larger nuclei were less deeply stained than the smaller ones, while hyperchromatic nuclei were seen in small numbers. In some regions elongated nuclei were sharply outlined. Mitotic figures were rare. The ependymal lining was not altered. A few ganglion cells revealed marked degeneration and fragmentation.

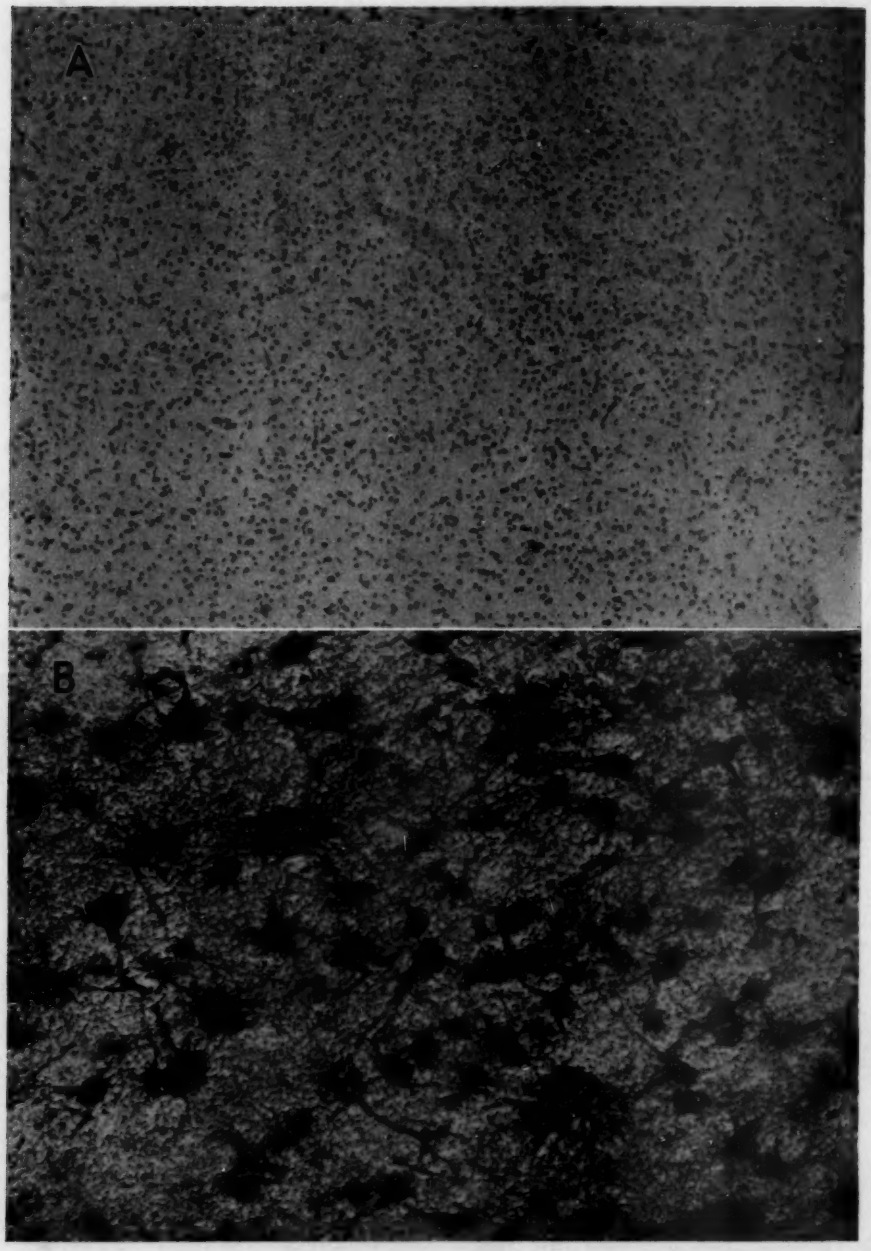

Fig. 3 (case 2).—*A*, photomicrograph of section from tumor of the region of the basal ganglion, stained with hematoxylin and eosin, revealing the structure of astrocytoma. *B*, photomicrograph of a section of the same tumor, stained with gold chloride. Astrocytes stand out clearly.

A recognizable fairly sharp border, for the most part, existed between the tumors and the surrounding tissue. In some areas, however, there was a gradual indistinct transition between them. The blood vessel walls were well formed, and were not thickened, and moderate perivascular edema was present.

Sections stained with Cajal's gold chloride revealed cells with fairly well developed multiple processes of varying lengths and with intensity in staining reaction (fig. 3). Frequently a single process could be demonstrated, with attachment to perivascular tissue. This was the predominating cell type; only an occasional cell was seen with one process or with bipolar processes. The cells were arranged in an irregular fashion, with occasional concentration about a blood vessel.

Sections from remote regions of the cerebrum, cerebellum, pons and cord showed no recognizable changes. The white matter contained a moderate number of glia nuclei. They did not appear more numerous than in sections taken from the same regions in the brains of children with no tumor. Rather wide zones about the tumors showed an increase in glial nuclei. With gold chloride stains these cells were seen to possess well formed, readily stained processes.

COMMENT

The gross appearance of all the lesions suggested neoplastic processes. The margins were fairly sharply limited, and the adjacent tissue was compressed. Naturally, this was less evident with the smaller tumors. Thus, in the second case this change was less evident than in the first. In the former some portions of the periphery faded out gradually into the surrounding tissue. It is to be remembered that the region near the ventricles is rather highly cellular in infants. However, comparisons of sections clearly demonstrated that in the brains of our patients the increase in cellularity involved a much wider zone than in brains with no tumor.

The microscopic study in both cases indicated a neoplastic process. In the first case the predominating cell was the spongioblast, and, less frequently, mature forms of glia cells were seen. In the second case the cells were mostly of the rather well differentiated type. In both cases the histologic appearance of the lesions on the two sides was similar. The clinical histories coincided with the histologic features. This is indicated by the degree of differentiation observed on histologic examination of the two types of tumors in the two cases. In the first case the history indicated a more rapidly growing tumor, as seen in the duration of symptoms of five months, as compared with the duration in the second case of twenty-two months.

The unusually interesting features in these cases are the identical location of the growths and the bilateral involvement without demonstrable connections between the two tumors. That in each case the tumors occurred in a young child and developed near the midline in the growth zone suggests that a congenital factor was at play in the development of the tumors. It suggests that cells in this zone had been diverted in the process of development, giving rise to abnormal growths. The factor or factors which cause such cells to be released from the physiologic influences usually leading to normal growth and differentiation are not known. Freeman [5] has noted an increase in the number

5. Freeman, W.: Arch. Neurol. & Psychiat. **14**:649, 1925.

of glia cells in the brains of patients with glial tumors, particularly on the side of the lesion. He expressed the belief that such observations indicated that a congenital factor was in existence. It is impossible for us to state whether or not in the cases here reported there was an increased number of glia cells outside the area of the tumor. There were no apparent differences on the two sides in either case. Since the tumors were bilateral, one would expect the two sides to be affected alike. We feel rather certain, however, that in regions farthest removed from the tumors no noticeable difference existed between the brains of our patients and brains in which no neoplasm was present in regard to the number of glia cells. The increase in glia cells surrounding the tumors can be regarded as a reactive gliosis, which is commonly seen about glial tumors.

It would be of interest to learn whether neuroglial tumors are more frequently multiple than other tumors. The most common multiple carcinomas are those of the skin. Neuroglia is of ectodermal origin. In several series of cases reported in the literature, multiple tumors of glial origin occurred 8 times in 532 cases. The 2 patients reported on here were among 24 who showed glial tumors at autopsy. This gives the frequency among 556 cases as 1.79 per cent. Schreiner and Wehr [6] observed that cases of multiple malignant growths occurred in 2.7 per cent of cases of 11,212 malignant lesions. If the number of cases of multiple malignant growths of the skin are subtracted, the percentage is 1.5. Warren and Gates [7] found that, on the basis of all statistics, the frequency of cases of multiple malignant growths is 1.84 per cent. In American statistical material it is 3.9 per cent. Bugher [8] obtained the figure of 3.1 per cent. The series of cases of tumor of the brain is too small to give the true indication of the frequency of multiplicity of glial tumors; however, the percentage in this series falls within the range just given. It is distinctly less than the percentage of cases of multiple tumors of the skin.

SUMMARY

Two cases of primary bilateral tumors of glial origin are reported. In one case the tumors were spongioblastic, while in the other they were astrocytic. The histologic structure was similar on the two sides in each case.

The occurrence in young children and the location of the tumors suggest the possibility that a congenital factor may have been of importance in their development.

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General Review

TISSUE CULTURE

WITH SPECIAL REFERENCE TO PATHOLOGY

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The main progress in the earlier stages of tissue culture has been achieved by investigators in the fields of experimental biology, physiology and cytology. Workers in other branches of science have since cooperated and introduced tissue culture into their laboratories as another auxiliary for the solution of their particular problems. Already books [1] have been written on this subject, to which I recommend reference for a study of the detailed technic of tissue culture. It is my aim in the present review to discuss the significance and usefulness of explantation with special reference to pathology.

HISTORY

During the eighties of the past century, Roux [2] was the first to succeed in obtaining growth of tissue outside the body. He kept a chicken embryo in a weak solution of sodium chloride and observed further development of the neural and intestinal canals. To this procedure he later gave the term explantation.

In 1897 Loeb [3] published observations concerning a suitable method for the cultivation of isolated particles of tissue.

Ten years later Harrison [4] communicated his investigations on the development of nerve fibers and experiments on the behavior of embryonic tissue isolated in clotted lymph.

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1. (*a*) Bisceglie, V., and Juhász-Schäffer, A.: Die Gewebezüchtung in Vitro, Berlin, Julius Springer, 1928. (*b*) Craciun, E. C.: La culture des tissus en biologie expérimentale, Paris, Masson & Cie, 1931. (*c*) Erdmann, R.: Praktikum der Gewebepflege, ed. 2, Berlin, Julius Springer, 1930. (*d*) Fischer, A.: Gewebezuechtung, Munich, Rudolph Müller & Steinicke, 1930.

2. Roux, W.: Gesammelte Abhandlungen über Entwickelungsmechanik der Organismen, Leipzig, Wilhelm Englemann, 1895, vol. 2, p. 247.

3. Loeb, L.: Ueber die Entstehung von Bindegewebe, Leucocyten und roten Blutkörperchen aus Epithel und über eine Methode isolierte Gewebsteile zu züchten, Chicago, M. Stern & Co., 1897.

4. Harrison, R. G.: Proc. Soc. Exper. Biol. & Med. **4**:140, 1907; J. Exper. Zoöl. **9**:794, 1910; Anat. Rec. **6**:181, 1912.

This was the fundamental work on which the modern method of tissue culture was based, elaborated and systematized during the past twenty-five years by Carrel,[5] who also introduced the use of blood plasma as a culture medium. Even today this medium is unsurpassed for routine explantation. The plasma provides the cells with a stable basis, which is a fundamental necessity for the ameboid movement and growth of the explanted cells. Loeb[6] pointed out that the migration and growth of cells occur only if the pseudopodia have a stable support, a phenomenon which he called stereotropism. In liquid mediums the cells become round and stop moving.

Since the establishment of the fundamental conditions nearly every other biologic method has been applied to explanted tissues. The experiments have been extended in two main directions: (1) the physiologic method, dealing with living or stained cultures in toto, and (2) the morphologic method, which in addition includes the examination of fixed and stained explants in serial sections.

GENERAL PHENOMENA OF EXPLANATION

Growth.—The main value of the explantation method is that it allows microscopic analysis of living material without reliance on fixed and stained preparations alone.

The earliest signs of life manifest themselves in a migration of the ameboid cells, which wander from the periphery of the explant into the plasma. Leukocytes, lymphocytes, monocytes and histiocytes are the advance guard. The cells show fast movement. They creep along the cover glass by means of their pseudopodia, while their nuclei and later their cytoplasm change. Within a few hours movement is first noted, and in from twelve to twenty-four hours a culmination point of the cell migration is reached. The explanted material itself undergoes certain changes. At the periphery the cells increase in size, regenerate and multiply by mitosis. The latent period of true growth may last for as long as three days, but normally within from twenty-four to forty-eight hours an outgrowth of cells from the explant is visible, as indicated by a new formation of its specific cells. These observations confirm the old thesis: *omnis cellula e cellula eiusdem generis.*

After three days an abundant growth outside the explant is seen, while the central area, beginning after one day, shows more and more signs of degeneration, necrosis and autolysis. The growing cells cause an increasing liquefaction of the plasma by the metabolism of the living

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6. Loeb, L.: Science **34**:414, 1911; **55**:22, 1922; Am. J. Physiol. **56**:140, 1921.

cells. The better the growth, the more liquefaction of the nourishing plasma. To support the stereotropism of the cells, transfers to fresh mediums must be made.

Cultivation of mesenchymal tissue is much easier than that of epithelial tissue from the technical angle. The earliest investigators stated the opinion that epithelial cells have a poor growth tendency, but more recent experiments have proved that epithelium may show even better growth than connective tissue elements. Simultaneously with good epithelial growth, liquefaction of the plasma spreads rapidly and widely, so that, according to the law of stereotropism, the growth of the cells stops. Thus, frequent passages must be made. The growth tendency of embryonic tissue is very good. Furthermore, the lower the differentiation of an animal, the better the explantability of its tissues. There exist certain differences in the fitness of the plasma used for the purpose of cultivation: rabbit, guinea-pig and chicken plasma are exceptionally suitable mediums, while human plasma on the whole is not. The reasons for this have not yet been fully clarified. Chemical factors seem to play an important rôle; Hirschfeld [7] obtained more favorable results by cultivating human blood in lipemic human plasma.

Growth and multiplication of cells take place by the way of mitosis. The maximal proliferation of cells is seen at the periphery, where mitotic figures are seen ten times more frequently than in the central portion. Olivo and Delorenzi [8] have accurately determined the rapidity of growth within a culture in accordance with the mitotic coefficient within the zone of migration, their topographic and chronological distribution and the duration of cell movement. Horning [9] emphasized that growth tendency depends on the rate of degeneration but that the rate of multiplication is not an index of the age of the subject from whom the tissue was taken. This specificity remains constant in the transfers,[10] no matter how often they may be made.

According to Maximow,[11] one may distinguish three different types of growth: (1) cytotypic growth, i. e., the cultivation of isolated cells (the example *κατ' ἐξοχήν* being growth of isolated mesenchymal cells); (2) histotypic growth, as shown by the membranous-like growth of epithelium, and (3) organotypic growth, as signified by the combined growth of different tissue elements in an organ-like arrangement, i. e., the combined growth of the epithelium and of the supporting mesenchymal stroma.

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TISSUES SUCCESSFULLY EXPLANTED

The following tissues have been successfully explanted: epithelium—embryonic iridal epithelium;[10] pure strains of lenticular epithelium[12] of chicken embryos; skin (Loeb,[13] Burrows,[14] Oppel[15]), demonstrating that growth originates from the basocellular layer[16] of the rete Malpighii, while epidermal spinal cells die; thyroid, with the formation of colloid substance (Burrows,[17] Ebeling[18]). Lung tissue,[19] with special reference to the alveolar phagocytes; hepatic tissue,[20] with the production of glycogen.[21] The liver of the rat shows a better growth tendency than that of the chicken. The epithelium of the bile ducts grows better than the hepatic cells themselves. The question whether epithelium of the bile ducts may become converted into hepatic cells[22] or vice versa has not yet been decided—a significant problem in the regenerative processes of hepatic tissue after retrogressive metamorphosis, as in yellow atrophy, cirrhosis and syphilis. Tissue culture favors a transformation of epithelium of the bile ducts into hepatic cells.

The behavior of pancreatic tissue[23] resembles that of tissue from the breast[24] and the salivary glands;[21a] their epithelium may become dedifferentiated, showing the appearance of atypically growing cancer cells. Epithelium from the pancreatic ducts grows better than do the cells of the Langerhans islets, which undergo dedifferentiation. The changes of various epithelial cells have been fully described and analyzed by Maximow[24] and Chlopin.[25]

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Gastro-intestinal tissue [26] of chicken embryos shows peristaltic movement from contraction of the muscularis. In bronchial tissue of the rabbit [27] the mucin cells disappear, or a conversion into indifferent epithelial cells occurs within nine days, with the cilia remaining alive. Tissue from the urinary bladder [28] and also the gallbladder [29] of newborn and adult animals has been explanted.

In experiments with tissue from the embryonic metanephros [30] of adult frogs [31] and guinea-pigs [32] an organotypic growth of the renal tissue [33] has been shown, the growth of the tubules taking place in a membranous-like fashion, but only the epithelium of the collecting tubules may survive, while that of the convoluted tubules undergoes degeneration.[32] Holmes and Watchorn [34] reported the production of ammonia and urea in cultivated renal tissue.

According to Ssipowsky [35] explanted adrenal glands reveal survival but no growth. In cultures of adrenal glands from chicken embryos Lewis and Geiling [36] observed not only survival of the tissue but also an increase in the amount of epinephrine produced, thus indicating a further production of the true hormone of the gland.

Growth of various tissues of the sex and generative apparatus has been studied: testicles and ovaries; [37] corpus luteum; [38] human [39] placenta [40] showing survival and growth, and finally the vitelline syncytium of the umbilical cord [41] in chicken embryos and of the wall of the umbilical vesicle.[42]

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Cultures of embryonic and adult nerve tissue,[43] both myelinic and amyelinic [43d] types, have revealed an insight into regeneration, degeneration and differentiation.

Ingebrigtsen,[44] in his studies of the cerebellum of the dog concerning degeneration and regeneration of the axis-cylinders, stated the conclusion that finally all the nerve cells and nerve fibers die. Marinesco and Minea [43d] pointed out that explanted nerve fibers of mammalians behave just like nerve fibers within the body. They demonstrated wallerian degeneration in vitro. In other experiments they reproduced degenerative lesions of nerve fibers, such as thickening and twisting, closely similar to presenile Alzheimer's disease. According to Mossa [45] the regenerative tendency of the central and peripheral processes of the spinal ganglions is nearly the same and is completely independent of the distance from the location of the ganglions. It is of importance for growth of nerve tissue to isolate it from the surrounding tissue (Levi [46]). He stated, furthermore, that there is a very high resistance of the neurite to microtraumatic influences.

Cerebrospinal fluid is a highly suitable medium for the cultivation of brain tissue. Martinović [47] succeeded in using it and showed survival of the cortex of the cat. Others [48] explanted brain tissue in the usual way and also reported survival. In brain tissue of 3 day chicken embryos Mihálik [49] observed differentiation into migrating macrophages and microglia cells. The glia cells show an epithelium-like membranous arrangement.[50]

Geiling and Lewis [51] explanted different parts of the pituitary gland: the pars intermedia did not reveal the property of raising the blood pressure, but there was a marked melanophore-stimulating effect when the cultivated tissue was tested on frogs. Tissue from the anterior part of the pituitary gland had no hormonal effect, but tissue from the posterior lobe mixed with fragments from the pars intermedia produced both a rise in the blood pressure and a melanophore-stimulating action. Connective tissue has been cultivated with special reference to the cytologic problems, origin and nature of the perivascular undifferentiated

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49. Mihálik, P.: Anat. Rec. **54**:157, 1932.

50. Olivo, O.: Arch. f. exper. Zellforsch. **4**:43, 1927; **8**:250, 1929.

51. Geiling, E. M. K., and Lewis, M. R.: Am. J. Physiol. **113**:534, 1935.

mesenchymal cells, reticulum cells, fibrocytes and histiocytes and their relationship to blood cells. Special attention was paid to the formation of collagenous and silver fibers.[52] Furthermore, the behavior of the vascular endothelium and that of the reticulo-endothelial system was investigated.[53] Rous and Beard [54] worked out a technic by which the Kupffer cells of the liver can be segregated by using their ability to store iron. The iron within the cells responds to magnetic influences, and the cells follow the magnetic attraction. Blood, bone marrow and all the hematopoietic and lymphopoietic organs are suitable for explantation.[55] Culture of tissue from the thymus [56] revealed that the small thymus cells behave like lymphocytes and may be separated from the cells of the reticulum. Occasionally concentric pearl-like whorls are formed. Thus the lympho-epithelial character of this organ can be elucidated. Mesothelial cells of serous membranes [57] and also thrombocytes [58] have been explanted.

Synovial membranes, though of mesenchymal origin, behave differently from ordinary connective tissue. Vaubel [59] demonstrated that they grow like epithelium, forming a syncytial network with spaces between, produced by plasmolysis, probably due to the action of proteolytic enzymes. Later a mucin-like substance is elaborated. Therefore these synovioblasts, as he called the newly grown cells, are closely allied to chondroblasts.

Growth of fat tissue is reported [60] and of cartilage, bone and periosteum of animal and human embryos. Cultivated chondroblasts of embryonic frontal bone, if grown slowly, produce cartilage from the fibrils [61] of the primary substance [62] and subsequent ossification.[63] Fell and Robinson [64] obtained an increase and a decrease of the phosphatase

52. Maximow,[19b] p. 232.

53. Silberberg, M., in Hirschfeld, H., and Hittmair, A.: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwarzenberg, 1933, vol. 1, pt. 2, p. 1034.

54. Rous, P., and Beard, J. W.: J. Exper. Med. **59**:577, 1934.

55. Bloom, W., in Hirschfeld, H., and Hittmair, A.: Handbuch der allgemeinen Hämatologie, Berlin, Urban & Schwarzenberg, 1933, vol. 1, pt. 2, p. 1254.

56. (*a*) Tschassownikow, N.: Arch. f. exper. Zellforsch. **3**:250, 1927. (*b*) Popoff: ibid. **4**:395, 1927.

57. Maximow, A.: Arch. f. mikr. Anat. **74**:525, 1925.

58. Delorenzi, E.: Arch. f. exper. Zellforsch. **17**:78, 1935.

59. Vaubel, E.: J. Exper. Med. **58**:63, 1933; Virchows Arch. f. path. Anat. **289**:670, 1933.

60. Burckhardt, L.: Arch. f. exper. Zellforsch. **16**:187, 1934.

61. Fischer, A.: J. Exper. Med. **36**:379, 1922.

62. Roulet, F.: Arch. f. exper. Zellforsch. **17**:1, 1935.

63. Studitsky, A. N.: Ztschr. f. Zellforsch. u. mikr. Anat. **20**:636, 1934; Arch. f. exper. Zellforsch. **13**:390, 1932. Fell, H. B.: ibid. **11**:245, 1931.

64. Fell, H. B., and Robinson, R.: Biochem. J. **23**:199 and 767, 1929.

activity during the growth of cartilage. Strangeways [65] kept cartilage alive in twenty-three passages. This material was reimplanted into animals. If the implant was removed after fourteen days, it was discovered converted into hyaline cartilage. The question arises whether a further differentiation of the cartilage takes place or whether under the influence of degenerating cartilage the connective tissue of the host undergoes metaplasia leading to the formation of hyaline cartilage.

Growth of cardiac tissue [66] with differentiation of muscle fibers has been observed; it retains a physiologic pulsation for a long time, and different pieces from the same subject, if explanted together, are capable of uniting and pulsating synchronously. This happens not only when the pieces are derived from the same heart but also when they are taken from different members of the same species, although not of different species.[67] Growth of skeletal muscle [68] also may occur.

Tissue infected with tuberculosis, glanders and leprosy has been studied, but material containing spirochetes is hardly cultivable.[69] Attempts were made to explant tissue obtained from a patient with Dupuytren's contracture,[70] with dubious result.

Tumor material has been grown: nasal polyps from human beings,[71] gliomas [72] and a hypernephroma of a guinea-pig.[73]

As regards cancer the principal work has dealt with the cultivation of the Ehrlich mouse-breast and the Flexner-Jobling type and also with material from human beings.[1]

Sarcomatous tissue has been repeatedly explanted, especially the Rous, Jensen and Crocker types of rat and human sarcomas.[1]

Cultivation of immature organs, in order to determine whether or not further differentiation will occur, has been attempted. Toe buds of chickens superficially injured showed irregular growth, while normal buds revealed a growth similar to that which is seen in the body.[74] Stoehr [75] reported further development of the primitive heart into four

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65. Strangeways, T.: Proc. Roy. Soc., London, s.B **100**:50, 1926; Technique of England, Tissue Culture "in Vitro," Cambridge, W. Heffer & Sons, Ltd., 1924.

66. Olivo, O.: Arch. f. exper. Zellforsch. **11**:245, 1931.

67. Fischer, A.: J. Exper. Med. **39**:577, 1924.

68. Renyi, G. L., and Hogue, M. J.: Arch. f. exper. Zellforsch. **16**:167, 1934.

69. Kast, C. C., and Kolmer, J. A.: Am. J. Syph. & Neurol. **17**:529, 1933. Bessemans, A., and de Geest, B.: Compt. rend. Soc. de biol. **114**:530, 1933.

70. Tuma, P.: Arch. f. exper. Zellforsch. **15**:173, 1934.

71. Skoog, T.: Acta oto-laryng. **19**:286, 1934.

72. (*a*) Kredel, F. C.: Am. J. Path. **4**:337, 1928. (*b*) Buckley, R. C.: ibid. **5**:467, 1929. (*c*) Russell, D. S., and Bland, J. O. W.: J. Path. & Bact. **36**: 273, 1933.

73. Roskin, G.: Ztschr. f. Krebsforsch. **35**:142, 1932.

74. Thomson, D.: Proc. Roy. Soc. Med. **7**:77, 1914.

75. Stoehr, P., Jr.: Naturwissenschaften **12**:337, 1924.

sections. In explanted otocysts [76] osteogenesis was seen; differentiation of the organ of Corti and of the endolymphatic duct was very slight or absent. According to Strangeways and Fell,[77] undifferentiated limb buds of 5½ or 6 day old chicken embryos show hypertrophy of the cartilage, which later becomes liquefied and cystic, but no further differentiation takes place. Fell and Robinson [78] investigated the amount of phosphatase and they observed an increase, with differentiation, during the first period but later a decrease, which proves that degeneration of cartilage and osteoid tissue had occurred. In explants of the embryonic eye of fowl Strangeways and Fell [79] demonstrated pigment formation and differentiation into the rods and cones of the retina, internal and external membrana granulosa, nerve cells, fibers of the lens and ciliary body. However, sooner or later the differentiation stops, and the outlook in this field does not seem very promising.

FINER MORPHOLOGY

The cytoplasm and the nucleus of a cell change shape all the time, movement taking place by means of plasmatic pseudopodia. Within the nucleus one or several nucleoli in various situations are noticeable: the membrane becomes retracted, thus leading to pleomorphic varieties of cells. The cytoplasm does not show a cell membrane in reflected light or in dark-field illumination,[80] and there is no distinct boundary between ectoplasm and endoplasm. Macklin [81] proved the existence of a centrosphere, which probably is caused by a slightly concentrated gel. Chambers [82] assumed that there is an invisible network in the endoplasm which contains the plasmatic substance in droplets.

Supravital Staining.—Using janus green, janus black and other supravital stains, the Lewises [83] and others demonstrated that the mitochondria have the shape of threads or small spheres and are invariably moving. The movement ceases only when the cell dies, a fact which is probably due to changes in the consistency of the cytoplasm. In contradistinction to earlier opinions, they noted that the mitochondria vary

76. Fell, H. B.: Arch. f. exper. Zellforsch. **7**:69, 1928.

77. Strangeways, T., and Fell, H. B.: Proc. Roy. Soc., London, s.B **99**:340, 1926.

78. Fell, H. B., and Robinson, R.: Biochem. J. **23**:14, 1929.

79. Strangeways, T., and Fell, H. B.: Proc. Roy. Soc., London, s.B **99**:60, 1926.

80. Fischer, A., in Abderhalden, E.: Handbuch der biologischen Arbeitsmethoden, Berlin, Urban & Schwarzenberg, 1927, vol. 5, p. 637.

81. Macklin, C. C.: Anat. Rec. **10**:225, 1916.

82. Chambers, R., in Cowdry, E. V.: General Cytology, Chicago, University of Chicago Press, 1924; Harvey Lectures, Baltimore, Williams & Wilkins Co., 1926-1927, vol. 22, p. 41.

83. Lewis, W. H., and Lewis, M. R.: Science **99**:330, 1914; in Cowdry, E. V.: General Cytology, Chicago, University of Chicago Press, 1924.

in number, position and form. During mitosis they become shorter, thicker and denser and then divide into equal parts, evenly distributed between both daughter cells. They represent fundamental structures of the living cell.[84] According to Carrel and Ebeling,[85] the variability of the mitochondria is connected with changes in the metabolism within the cells. Conversion of the mitochondria into other cell elements or granules, such as neutral red or pigment granules, fat droplets or vacuoles, is not seen and is denied by the majority of investigators.

Though further details have been elucidated, one cannot yet determine their real nature and significance. The combined application of two supravital stains, chiefly neutral red and janus green, revealed besides mitochondria the presence of a special variety of droplets which are stainable with neutral red or osmic acid. Some authors have stressed the connection of these droplets with the elements of the Golgi apparatus. These "neutral red granules" change in size and number and seem to be identical in some instances with the secretory apparatus.[86] In phagocytes they increase with hyperfunction of the cell. The substance as demonstrated by neutral red is probably fluid [87] and identical with the osmophilic material.

Vital Storage.—The most commonly used dyes are carmine and pyrrhol blue, which give approximately the same results, but other dyes also are used. Different preparations reveal storage to a variable degree. Wallbach [88] investigated twenty-two vital dyes and analyzed their ingestibility. As a rule, one is justified in saying that the phenomena of vital storage in vitro follow the same principles as that within the living body. Only macrophages, e. g., histiocytes, take part in the vital storage, while microphages, i. e., leukocytes, do not. Fibrocytes and undifferentiated mesenchymal cells show but a slight tendency to store the dye within their cytoplasm and retain it at all only if large doses are applied. In these particular cases a fine granular phagocytosis may be observed within the fibrocytes and undifferentiated mesenchymal cells, but it is different from that in the histiocytes.

The vital dyes are added either to the tissue before explantation or to the drop of clotted plasma. Both methods are feasible. Phagocytosis within the macrophages becomes evident in from twenty-four to forty-eight hours after explantation and depends on the vitality, migration and growth potency of the cells. The leukocytes show a similar function, taking up microparticles, such as cocci or india ink.

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84. Levi, G., and Bucciante, L.: Anat. Anz. (supp.) **66**:263, 1928.
85. Carrel, A., and Ebeling, A. H.: J. Exper. Med. **44**:285, 1926.
86. Evans, H., and Scott, K.: Contrib. Embryol. **10**:1, 1921. Richardson, K. C.: Arch. f. exper. Zellforsch. **16**:100, 1934.
87. Rumjantzew, A.: Arch. f. exper. Zellforsch. **7**:107, 1928.
88. Wallbach, G.: Arch. f. exper. Zellforsch. **10**:383, 1931.

On the other hand, cells of the mesonephros [89] and many types of epithelial cells, such as those from the cornea, urinary bladder, gallbladder, tongue and intestines,[48] likewise may show storage of vital dyes, a faculty which is not observed within the body. An explanation might be sought in the fact that the contact of epithelial cells and dyes is closer and more direct in culture than within the body.

METABOLISM

Flask and cover glass cultures have been analyzed. Erdmann [90] and her collaborators, Warburg and Kubowitz [91] and others [92] have shown that metabolism is always higher in explanted tissues than in normal tissues. This is easily comprehensible, because the explants are living under the richest conditions. Each tissue, even under aerobic conditions, shows marked glycolysis.[93] The great amount of lactic acid present is perhaps due to injury of the cells, not to the rapidity of their growth.[94]

The results concerning fat metabolism are not entirely conclusive, as they differ widely.[95] These differences seem to depend on chemical conditions within the nutritive solutions and on inherent growth energies of the particular tissue.

RETROGRESSIVE METAMORPHOSIS

Endogenous Pigments.—Studies of melanin,[96] especially in embryonic epithelium of the choroid, retina and iris and in the cell layers of the rete Malpighii of the skin, is elaborated within the cells independently of nuclei and mitochondria, becoming visible as fine colorless particles but only if there is sufficient time for complete differentiation, i. e., if their growth and proliferation are not stimulated by growth-promoting extracts. In other words, the production of melanin is a physiologic function of certain fully matured and differentiated cells. The mechanism of conversion of those colorless particles into melanin has not been clarified.

Pigments derived from the disintegration of hemoglobin have been produced, and extracellular dissolution of the red particles can be easily seen. This soluble, blood-colored material, which is phagocytosed by the macrophages, is stored within their cytoplasm in granular form.

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89. Chambers, R.: Proc. Soc. Exper. Biol. & Med. **32**:1199, 1935.
90. Erdmann, R., and others: Arch. f. exper. Zellforsch. **3**:395, 1927.
91. Warburg, O., and Kubowitz, F.: Biochem. Ztschr. **189**:242, 1927.
92. Lipmann, F.: Biochem. Ztschr. **244**:177, 1932.
93. Demuth, F., and Meier, R.: Biochem. Ztschr. **212**:339, 1929.
94. Demuth, F.: Arch. f. exper. Zellforsch. **11**:98, 1931.
95. Rix, E.: Arch. f. exper. Zellforsch. **13**:517, 1933.
96. Smith, D. T.: Bull. Johns Hopkins Hosp. **31**:239, 1920. Fischer,[1d] Bisceglie, V.: Ztschr. f. Zellforsch. u. mikr. Anat. **16**:228, 1932.

In unstained and living preparations hemosiderin manifests itself as a dark yellowish or brownish refractile mass, which gives all the characteristic microchemical reactions for iron. The slower the growth tendency of a culture, the more pronounced is its tendency for hemosiderophagocytosis.

The formation of iron-free pigment in explanted hepatic tissue of rabbits has been described by Mitsuda,[97] but he did not classify it chemically. Niven [98] demonstrated recently the formation of hematoidin from the hemoglobin of mammalian erythrocytes, and his work has been supported by Balogh, Sümegi and Csaba,[99] who cultivated various tissues of chicken and guinea-pig embryos for three or four days and succeeded in the reproduction of bilirubin in cells of the spleen, brain, spinal cord, pericardium, iris and medulla of the adrenal glands, but they did not with those of the heart, skeletal muscle, costal cartilage and walls of the veins. The bilirubin reaction was tested by the van den Bergh method. Their findings support the theory of the extrahepatic formation of bilirubin. They observed, further, that bilirubin was more abundantly elaborated within the spleen than in any other organ. They thus attributed this function to the reticulo-endothelial apparatus.

The formation of yellow pigment during the decomposition of hemoglobin has been reported repeatedly.[100] Mitsuda [97] could not detect a genetic relationship between it and the fatty elements of the cells, and if his conclusions were correct, the brown fuscous pigment, hemofuscin or lipochrome, could not be due to disturbances of fat metabolism.

Exogenous Pigmentation.—Dust and particles of coal, stone, quartz, iron, basalt, chalk, glate and mountain-crystal added to explants raised hopes of a contribution to the pathology of pneumonoconiosis.[101] The experiments have invariably shown that the particles are digested mainly by the macrophages. Such cells in tissue from patients with silicosis, siderosis and chalicosis are observed to be identical with the alveolar phagocytes. Even cells showing lipoid infiltration after a longer period of growth retain their phagocytic capacities. Various substances show differences in the quantity of material absorbed: Coal is phagocytosed

97. Mitsuda, F.: Virchows Arch. f. path. Anat. **248**:91, 1924.

98. Niven, J. S. F.: J. Path. & Bact. **41**:177, 1935.

99. Sümegi, S.; Csaba, M., and Balogh, E.: Virchows Arch. f. path. Anat. **293**:320, 1934.

100. Doljanski, L., and Koch, O.: Virchows Arch. f. path. Anat. **291**:379, 1933. Policard, A.; Doubrow, S., and Boucharlat, M.: Bull. d'histol appliq. à la physiol. **6**:371, 1929.

101. (*a*) Henke, F., and Silberberg, M.: Verhandl. d. deutsch. path. Gesellsch. **24**:125, 1929. (*b*) Lauche, A.: ibid. **26**:107, 1931.

more readily than other material.[101b] In spite of simultaneous storage of two different forms of dust, no essential differences have been established.

Colloid.—In 1913 Burrows [17] reported colloid formation in explanted thyroid tissue, but only if the cultures were not transferred for a long period. Ebeling [18] kept cultures of thyroid tissue of chicken embryos alive for seven months, and colloid formation was noticeable after four months' growth. It took place when the growth of the thyroid tissue was not so vigorous. Should further observations support this fact, they would demonstrate that growth occurs in reverse proportion to the function of the tissues.

Fat.—Biedermann and Hoefer,[102] in cultivating material from a patient who showed the Schüller-Christian syndrome, showed growth of xanthomatous cells containing lipoids of double-refracting character, thus giving proof that cholesterol may originate from disintegrating red blood corpuscles. However, the newly grown xanthomatous cells may retain their physiologic and pathologic qualities, and the results obtained as to fat and lipoids [103] have made no conclusive contributions to the important problem of "fatty degeneration." Investigations in vitro could settle the question whether fatty degeneration does occur or whether there is merely a fatty infiltration of the cells. In cultures a saturation of cells runs parallel with the increasing age of the cells and a decrease of their growth tendency. This fact has not been given proper consideration, though Foot [104] in 1912 stressed the fact that no fatty degeneration occurs. Workers in this field can observe without difficulty that the cells are alive, though the cytoplasm is filled with lipoids. The nuclei are well preserved, and at every stage mitosis is seen. Krontowski [105] pointed out that fat within the cytoplasm of explanted cells appears during autolysis; it appears under poor cultural conditions, as with fatty plasma, and can disappear again on removal of the obnoxious causes. Thus it may be reasonable to abandon the ancient term fatty degeneration. The further outlook in settling this question is certainly encouraging.

Glycogen.—Synthesis in cultivated hepatic cells was demonstrated by Nordmann [21a] but only under favorable conditions. The production of glycogen is in proportion to the rapidity of growth and is independent of the medium used. The glycogen is dissolved within the cells. Fat does not influence or prevent this growth, but reducing the rate of growth prevents glycogen synthesis. According to these investigations, the rate of growth forms a measure of the metabolism. As previously

102. Biedermann, W., and Hoefer, K.: Arch. f. exper. Zellforsch. **10**:93, 1930.
103. Wylegschanin, A. J.: Ztschr. f. Zellforsch. u. mikr. Anat. **21**:342, 1934.
104. Foot, N. C.: Beitr. z. path. Anat. u. z. allg. Path. **53**:446, 1912.
105. Krontowski, A. A.: Ergebn. d. Physiol. **26**:370, 1928.

noted, with pigmentation or colloid formation a dissociation between the function of a cell, on the one hand, and the rapidity of growth, on the other hand, was found, and hence in this concern the relationship has yet to be decided. The problem is still more interesting, as Kapel [48b] demonstrated a synthesis of glycogen in explanted intestinal epithelium on adding dextrose to the culture medium. On the other hand, Herzog [106] saw an abundance of glycogen in cultivated epithelial cells of embryonic intestine of the guinea-pig without the addition of dextrose. The question now arises as to whether or not the production of glycogen in the cultures of liver tissue is a specific function of the

Fig. 1.—Control tissue after five days of cultivation.

hepatic cells. But further investigation must decide whether a productive glycogen synthesis occurs in cells other than those of the liver, whether it is a sign of so-called glycogen degeneration or whether the observations of glycogen production in intestinal epithelium were merely the result of artificial conditions within the explants.

Keratinization.—Drew [107] concluded that dedifferentiation of epithelium is delayed or does not take place in the presence of connective tissue, probably owing to some specific action of the connective tissue.

106. Herzog, G., and Fischer, A.: Verhandl. d. deutsch. path. Gesellsch. **26**: 9, 1931.

107. Drew, A. H.: Brit. J. Exper. Path. **3**:20, 1922.

Fischer [108] pointed out that organization is not due to any specific influence of the associated connective tissue, as in old cultures fragments of tissue became thick when they suffered from lack of oxygen and nourishment, and thus keratinization of epithelium occurred. The main question arises as to whether such conditions indicate a process of differentiation or one of degeneration.

Calcification.—Calcification has been reported [109] in cultures of cartilage and osseous tissue, the interstitial tissue taking up the calcium and lime salts, leading to an impregnation with chalky material and finally complete calcification.

Fig. 2.—Myositis ossificans. Tissue after five days of cultivation.

On explanting material from a patient with generalized progressive myositis ossificans, I observed calcification when the cartilage underwent retrogression. In comparison with normal cartilage, the resistance of this cartilage was distinctly greater. Growth of connective tissue occurred, but not of cartilage or bone. However, the cartilage remained alive after five days of cultivation, and only after eight or nine days (two transfers) did it degenerate, as indicated by vacuolation and dissolution of the cells. Associated with these retrogressive changes a marked precipitation within the interstitial tissue became evident,

108. Fischer, A.: J. Exper. Med. **39**:585, 1924.
109. Demuth, F.: Monatschr. f. Kinderh. **38**:79, 1928.

increasing from day to day, so that after ten days of cultivation, large areas of the explant were completely calcified. On the other hand, in the control tissue degeneration of cartilage started after three or four days, and only a little calcification was seen. It seems, therefore, that calcification depends on the preexisting amount of calcium in the tissue.

Hueper and Russell [110] dealt with the precipitation of calcium and alkalinization in aerobic cultures. Brand and Nauck [111] demonstrated calcification under the influence of vitamin D and the parathyroid hormone.

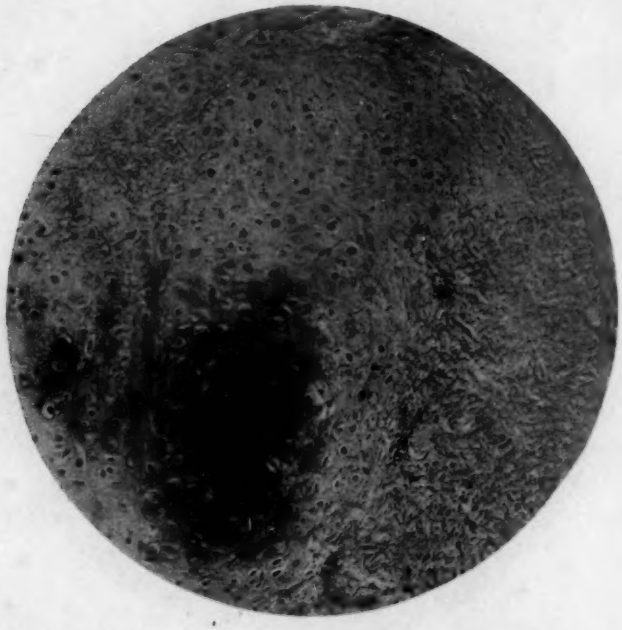

Fig. 3.—Myositis ossificans. Tissue after ten days in vitro.

INFLUENCES ON GROWTH

Tissue cultivation has provided a suitable method for the analysis of the complicated principles of growth, since the growth of isolated cells and groups of cells can be followed either in connection with nerves or independently. The experiments diverge in different directions as the result of (1) the effect of physical influences and (2) the effect and mechanism of chemical and pharmacologic agents.

Physical Influences.—The oftener a culture is cut the better its growth. The phenomena in vitro compare with the healing process of

110. Hueper, W. C., and Russell, M.: Am. J. M. Sc. **186**:383, 1933.

111. von Brand, T., and Nauck, E. G.: Arch. f. exper. Zellforsch. **14**:276, 1933.

wounds within the body. Fischer [1d] stated that wounds may elaborate certain hormonal substances, which he called desmones, being identical with Carrel's trephones, Caspari's necrohormones and Haberlandt's wound hormones. These substances are supposedly produced by regeneration, necrosis and necrobiosis of cell material and may have a growth-promoting influence on neighboring tissue. The parallelism between regeneration in vitro and the growth of tumors under these influences and also within the body supports the view that regeneration and tumor growth may be similar processes fundamentally; [112] living or dead tissue added to the plasma has an inhibiting effect on liquefaction. Furthermore, it is a general rule that two or more particles of tissue explanted within the same culture reveal a better growth tendency than if explanted singly.

Heat.—Kokott [113] noted a diminution of the superficial tension of fibrocytes so that they become round. The work of Pincus and Fischer [114] showed no lethal effect in explanted chick osteoblasts exposed to supranormal temperatures of 42 and 44 C. for unlimited periods. An exposure to 47 C. for one hundred and forty-five minutes, to 50 C. for six minutes or to 52 C. for three and one-half minutes killed the cultures. Therefore, a definite inhibition of growth occurs after varying exposures to all the temperatures from 44 C. upward. Heaton [115] added heated embryonic juice to cultures and reported an inhibition of the growth of connective tissue in favor of that of epithelium. Doljansky [116] had a similar experience when he cultivated hepatic tissue in warm extract. The investigations of Soares [117] demonstrated the same fact when the cultures were exposed to nitralight for certain periods.

Should these observations concerning the influence of heat hold good, the outlook would be encouraging for a more favorable cultivation of epithelium, which as yet is far from perfect.

Cold.—An enormous resistance of cells to cold is noticeable, even to freezing. Bucciante [118] kept tissues and strains of cells alive at temperatures as low as — 10 C.

Exsiccation.—A striking resistance of cells and tissues has been found. Morosow [119] dried the heart of the axolotl, reducing it to about 70 per cent of its substance, kept it at 4 C. for one hundred hours and yet succeeded in cultivating it. Pieces from the chick or human heart

112. Fischer, A.: Virchows Arch. f. path. Anat. **279**:91, 1930.
113. Kokott, W.: Ztschr. f. Zellforsch. u. mikr. Anat. **12**:327, 1930.
114. Pincus, G., and Fischer, A.: J. Exper. Med. **54**:323, 1931.
115. Heaton, N. B.: J. Path. & Bact. **32**:565, 1929.
116. Doljansky, L.: Compt. rend. Soc. de biol. **101**:754, 1929.
117. Soares, P. J.: Compt. rend. Soc. de biol. **116**:732, 1934.
118. Bucciante, L.: Monitore zool. ital. **40**:313, 1929.
119. Morosow, B. D.: Arch. f. exper. Zellforsch. **10**:157, 1930.

are able to survive dehydration to about 80 per cent. Brain tissue of chick embroys exsiccated to 90.7 per cent showed growth of the nervous substances, though the dried material had been kept in a refrigerator for one hundred and forty-four hours previous to explantation.

Irradiation.—Cultures of chick fibrocytes or nerve fibers exposed to ultraviolet radiation [120] showed inhibition of growth. Similar results were obtained by exposure to hertzian [121] and Gurvich rays.[122]

The important question of the influence of radium cannot be considered as unanimously solved so far. Fibrocytes have been chiefly employed in the test. Stenstrom and King [123] investigated the effect on lymph nodes. Love [124] pointed out that sensitivity to radium depends on the distance of a cell from its maturity. If the distance from division is less than three hours, the sensitivity is constant; but if it is more than three hours, there is a noticeable decrease. The effect is indicated by a reduction in the number of mitotic figures, probably due to special injuries to the cells, which normally would have undergone division. This first phase, characterized by a decrease in the number of mitotic figures, is later on followed by a superimposition of complete or almost complete recovery after a temporary inhibition of growth. The individual rays were examined also as to their specific activity: alpha [125] and gamma [126] rays. The characteristic fall and recovery of the number of mitotic figures in normal fibrocytes depend on the length and strength of the exposure. A cell in the process of division, at the onset of radiation, proceeds in a normal fashion to complete mitosis. Abnormal mitosis (shrunken and clumped chromatin) is seen shortly after irradiation, as a result of damage to the consistency of the spindle substance; but no injury to the mitochondria or nucleoli is observed. Cells of rat sarcoma show a similar behavior if exposed to 5, 16 and 50 millicurie hours. However, the irradiated cultures are unable to live when transferred to a fresh medium. Macrophages are evidently less affected by irradiation than tumor cells. No direct stimulation has been found, if measured by the consumption of oxygen. According to Flinn, Victor, Stillman and MacDonald [127] epithelial cells respond in the same manner but seem to be more susceptible to variations in temperature and the

120. Mayer, E.: Virchows Arch. f. path. Anat. **277**:363, 1930. Lazarenko, T., and Benenson, M.: Arch. f. exper. Zellforsch. **13**:413, 1932.

121. Roffo, A., Jr.: Néoplasmes **11**:257, 1932.

122. Zakrzewski, Z.: Arch. f. exper. Zellforsch. **14**:471, 1933.

123. Stenstrom, W., and King, J. T.: Proc. Soc. Exper. Biol. & Med. **31**: 909, 1934.

124. Love, H.: Arch. f. exper. Zellforsch. **11**:435, 1931.

125. Bucciante, L., and Foà, A.: Arch. f. exper. Zellforsch. **15**:190, 1934.

126. Whitman, W. G.: Am. J. Cancer **17**:932, 1933.

127. Flinn, F. B.; Victor, J.; Stillman, N., and MacDonald, D.: Am. J. Cancer **22**:351, 1934.

consistency of the plasma than mesenchymal cells. They do not recover from injury as rapidly as the mesenchymal cells. Radium applied in doses of 3.3×10^{-6} milligrams has no lethal effect, even if the tissue had already passed through twenty-nine subcultures. Radium is more lethal if all three rays come into intimate contact with the tissue than if the tissues are exposed to the individual rays.

From the histologic angle no definite statement as to the irritative and lethal doses has been made. Irritation and stimulation were not observed, though sometimes retrogressive phenomena were described, even giant cell formation.[128]

The results obtained with x-rays correspond to those obtained with radium. Cox [129] and Love [130] stressed that the premitotic stage is the sensitive phase. Within the first twenty-four hours no morphologic changes are visible, but a certain delay of growth on the part of the fibrocytes is noted after two days' cultivation. Tannenberg and Heeren [131] recorded qualitative changes, especially degeneration, but only after two or three transfers, i. e., from four to seven days after irradiation.

Tschassownikow [132] observed that the effect of x-rays on cultivated thymus tissue was shown by a diminution or absence of the small thymus cells after two days, but abundant growth of the epithelium took place. The Hassall corpuscles died. These observations support the view that within the thymus are two different categories of cells, namely, cells of lymphocytic and cells of epithelial types, a fact which is noteworthy in regard to the histogenesis of lympho-epithelial tumors.

The influences of electric phenomena and currents on the stimulation of growth and their relations to organization were examined by Huzella.[133]

Photodynamic influences have been reported:[134] prevention of the growth of epithelial cells sensitized by eosin A, erythrosin, methylene blue and acriflavine hydrochloride when exposed to full light or to darkness. Nonfluorescent chemicals, however, do not sensitize the cells to light.

Chemical Influences.—The intensity of growth, besides many other factors, depends on the hydrogen ion concentration of the culture mediums. Individual tissues vary in having their optimum growth at

128. Torrioli, M., and Giusti, M.: Policlinico (sez. med.) **41**:381, 1934.
129. Cox, S. F.: Arch. f. exper. Zellforsch. **11**:121, 1931.
130. Love, H.: Arch. f. exper. Zellforsch. **11**:132, 1931.
131. Tannenberg, J., and Heeren, J.: Klin. Wchnschr. **10**:2208, 1931.
132. Tschassownikow, N.: Ztschr. f. Zellforsch. u. mikr. Anat. **8**:251, 1929.
133. Huzella, T.: Arch. f. exper. Zellforsch. **15**:250, 1934.
134. Roffo, A. H.: Ztschr. f. Krebsforsch. **35**:415, 1932. Umeda, T., and Shibata, S.: Arch. f. exper. Zellforsch. **7**:344, 1928.

different concentrations. For instance, Mihálik [135] found that nerve cells migrate and grow at a p_H of from 5.8 to 7.8, their optimum concentration being between 6.6 and 6.8. In addition, the explanted tissue itself has the tendency to regulate the p_H to this point. Should the p_H be too low, it increases; if too high, it becomes lowered, owing to metabolic conditions within the culture.

Considering these facts, one may easily understand why authors have obtained variable results. Reviewing the problem of tissue culture from the standpoint of pathology, one cannot include a discussion of the many pharmacodynamic questions involved. Mention can be made only of some substances investigated as to their effect on growth: Embryonic juice counteracts the degeneration of the cells and acts therefore as a growth-promoting agent. Heparin, used in the more recent technic for preparation of the plasma, has a growth-diminishing effect. Fischer and Parker [136] demonstrated that fibrocytes multiply more abundantly in plain serum than in heparinized serum and again more abundantly in heparinized serum than in heparinized plasma. At later periods of growth, especially after passages, the differences become less marked, and normal growth occurs. Yet, the first effect of heparin is invariably injurious, the degree of damage depending on the nature of the cells and the age of the animals from which the plasma was prepared. Proliferation and differentiation are regulated by an interaction of prothrombin and antiprothrombin, i. e., heparin; the former acts as a growth-stimulating agent; the heparin, through its inactivation of prothrombin, acts as an inhibiting factor. The addition of heparin to plasma, therefore, would favor differentiation by decreasing the proliferative activity.[1d]

The amount of glutathione runs parallel to the intensity of growth. It has a growth-stimulating effect.[137]

Ethyl alcohol in dilutions of from 0.1 to 1 per cent stimulates the proliferation of fibrocytes but completely prevents the emigration of leukocytes and histiocytes. Propyl, isopropyl and butyl alcohol are more injurious.[138] Pilocarpine [139] in dilutions of from 0.5 to 2 per cent, chloral, morphine, strychnine and nicotine [140] stimulate growth, while epinephrine [141] has an inhibitory effect. There is good reason to assume that some of these chemicals exert their effect by vagotonic and others by sympathicotonic irritation. Some of these reports were made many

135. Mihálik, P.: Anat. Rec. **54**:149 and 157, 1932.

136. Fischer, A., and Parker, R. C.: Arch. f. exper. Zellforsch. **8**:325, 1929.

137. Baker, A.: Science **68**:459, 1928. Ephrussi, B.: Compt. rend. Acad. d. sc. **192**:1763, 1931.

138. Singer, E., and Hoder, F.: Arch. f. exper. Zellforsch. **8**:443, 1929.

139. Baroni, N.: Compt. rend. Soc. de biol. **101**:885, 1929.

140. Sanguineti, L. R.: Riv. di pat. nerv. **19**:257, 1914.

141. Cervello, V., and Levi, G.: Arch. di fisiol. **15**:219, 1917.

years ago, so that in order to settle this question it would be desirable to study the influence of the different drugs in accordance with the more recent views. The fact that the cells of the buffy coat can be cultivated is thus invaluable. In this way one can exclude any participation or intermediation of nerve fibers on growing cells. Dr. Dreyer and I in collaboration started experiments in this direction. Our observations so far reveal that a direct influence on growing cells and tissues may be exerted. Accordingly, one may decide whether the biologic phenomena are caused and regulated by primary irritation of the nerves (Ricker [142]) or by primary cellular irritation (Virchow).

The effects, furthermore, differ according to the tissue on which the investigations are carried out. Demuth [143] performed experiments concerning the influence of different substances on fibrocytes, iridal epithelium of chickens and Ehrlich's mouse carcinoma. Calcium produces a decrease in the size of explanted cells proportional to the amount administered and its ionization. Sodium bicarbonate, on the other hand, producing alkalinization, antagonizes the effect of calcium and entirely prevents the growth of cancer cells. Potassium acts in a similar manner, while carbon dioxide reveals an even stronger counteraction. Hydrochloric acid causes a diminution of cells, while sodium hydroxide increases them. Sodium mono-hydrogen phosphate effects a decrease in size of the cells; sodium di-hydrogen phosphate diminishes the size of only cancer cells and prevents their growth even more definitely than the primary phosphate. Thus a disturbance in the phosphate metabolism of cancer cells can be proved. With the application of magnesium and lactic acid no effect was noted; dextrose is a strong growth-promoting agent for cancer cells, probably on account of their fermentation. Investigations concerning the pharmacologic mechanism of chemicals were started recently, and the results have been contradictory: The stimulating effects of epinephrine and digitalis [144] on the function of the heart were examined. Roffo and Calcagno [145] studied the behavior of tissues under the influence of various vanadates—sodium, potassium, ammonia, lithium, rubidium, cesium, magnesium and calcium.

Another group of experimenters, working with antiseptics, have tried to control and standardize the effects and to find out the effective bactericidal dosage without damaging the cells. German [146] and Lambert and Meyer [147] investigated the relative toxity of strong protein silver

142. Ricker, G.: Die Relationspathologie, Berlin, Julius Springer, 1922.

143. Demuth, F.: Verhandl. d. deutsch. path. Gesellsch. **26**:95, 1931.

144. Varga, A.: Arch. f. exper. Zellforsch. **11**:312, 1931. Csaba, M., and Németh, L.: ibid. **11**:305, 1931.

145. Roffo, A. H., and Calcagno, O.: Ann. de physiol. **7**:649, 1931.

146. German, W. J.: Arch. Surg. **18**:1920, 1929.

147. Lambert, R. A., and Meyer, J. R.: Proc. Soc. Exper. Biol. & Med. **23**: 429, 1926.

and albargin, alcoholic iodine, mercury preparations, acriflavine, neoarsphenamine, hexylresorcinol, gentian violet and tri-nitrophenol. Buchsbaum and Bloom [148] analyzed the effect of phenol, metaphen, merthiolate and mercurochrome; Hogue [149] analyzed the germicidal power of bismuth iodide, carbarsone, a pentavalent arsenical compound (the sodium salt of phenyl-β-aminopropronanide-para-arsonic acid) and vioform, while Salle and Lazarus [150] compared the resistance of bacteria and embryonic tissue to hexylresorcinol, merthiolate, iodine and mercury preparations. Potassium iodide in dilutions of 1:40,000 and 1:80,000 injures cytoplasm; it coagulates and becomes vacuolated, and the mitochondria disappear.[151]

Bile,[152] cholesterol and other substances that decrease the superficial tension of cells induce a better growth of epithelial cells than do the ordinary mediums.

Plasma obtained from tuberculous rabbits three or four months after inoculation with bovine bacilli exerts a growth-inhibiting effect on explanted fibrocytes of the same species, while that obtained after four months shows a growth-stimulating influence.[153] The inhibiting factor may be linked with lipemia and the stimulating effect with leukocytosis. Lipemia therefore should have a definite influence on growth.

The experiments concerning the effect of hormones is still in an early phase. The growth-preventing influences of the gonadotropic principle from the urine of pregnant women and a spleen extract [154] on fibrocytes and cancer cells is noted. Parathyroid hormone stimulates calcification within growing tissues.

A growth-stimulating effect of vitamins B [155] and D [156] has been reported. Juhász-Schäffer [157] noticed the growth-stimulating influence of vitamin E. Wheat oil added to cultivated tissues produces a pronounced effect, even if the vitamin is inactivated. Furthermore, wheat oil is assimilated by the growing cells. The results are reliable, as the author compared the growth in cultures with that under ordinary conditions.

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148. Buchsbaum, R., and Bloom, W.: Proc. Soc. Exper. Biol. & Med. **28**: 1060, 1931.
149. Hogue, M. J.: Am. J. Trop. Med. **14**:443, 1934.
150. Salle, A. J., and Lazarus, A. S.: Proc. Soc. Exper. Biol. & Med. **32**: 1119, 1935.
151. Lewis, W. H.: Am. J. Anat. **28**:431, 1921.
152. Katzenstein and Knake: Ztschr. f. Krebsforsch. **35**:415, 1932.
153. Swift, H. F.; Moen, J. K., and Vaubel, E.: J. Exper. Med. **60**:149, 1934.
154. Ludwig, F., and von Ries, J.: Helvet. med. acta **2**:624, 1934.
155. Okamoto, K.: Mitt. d. med. Gesellsch. zu Tokio **48**:27, 1934.
156. Hosomo, G., and Navisawa, S.: Tr. Jap. Path. Soc. **21**:121, 1931.
157. Juhász-Schäffer, A.: Virchows Arch. f. path. Anat. **281**:35, 1931.

INFLAMMATION

Hematogenous factors, histogenesis and the reaction of the vascular endothelium must be considered.

Hematogenous Reaction.—Experiments carried out with the buffy coat elucidate the transformation of the different varieties of cells. Studies of blood of patients with leukemia—acute and chronic, lymphatic and myelogenous [158]—have invariably shown a transformation of lymphocytes into polyblasts and later into fibrocytes. Leukemic cells are of immature pathologic types. Hence the cultures of normal peripheral blood [159] or of lymph [160a] are more conclusive. It was proved once again, in contradistinction to E. Ziegler's view, that polymorphonuclears have no ability for further differentiation. Maximow's views on lymphocytes have been supported: Small and medium-sized lymphocytes may become converted into polyblasts, into histiocytes and finally into fibrocytes, thus playing an important rôle in elaborating the granulation tissue. Monocytes undergo the same changes.[160b] Explantation of the buffy coat without the addition of embryonic juice [159e] gave the same results, though growth and conversion were delayed. These results are important in obviating the objection that the embryonic juice may have contained any connective tissue cells. Only a limited number of lymphocytes degenerate, but the majority survive, showing conversion into polyblasts. Monocytes cannot be the only source for the production of polyblasts, if one considers that in the blood of rabbits and guinea-pigs only 2 per cent of monocytes are present. The blood of rats, which was used by Seemann,[161] is not very suitable for these investigations on account of the high percentage of "monocytoid lymphocytes." Aschoff and Seemann [161] have recently agreed that a conversion of the monocytoid lymphocytes into polyblasts may occur, certainly a further strong support for the view of a transformation of the lymphocytes at large, because with a precise cytologic technic a monocytoid structure can easily be demonstrated in the vast majority of the lymphocytes. In

158. Timofejewsky, A. D., and Benewolenskaja, S.: Arch. f. exper. Zellforsch. **8**:1, 1929. Silberberg, M., and Voit, K.: Deutsches Arch. f. klin. Med. **171**:110, 1931. Pierce, M.: Arch. Path. **14**:295, 1932.

159. (*a*) Maximow, A.: Arch. f. exper. Zellforsch. **5**:169, 1927-1928. (*b*) Bergel, S.: ibid. **9**:269, 1930. (*c*) Carrel, A., and Ebeling, A. H.: J. Exper. Med. **36**:365, 1922; **38**:513, 1923; **44**:285, 1926. (*d*) Awrorow, P. U., and Timofejewsky, A.: Virchows Arch. f. path. Anat. **216**:184, 1914. (*e*) Katzenstein, R.: ibid. **281**:172, 1931.

160. (*a*) Bloom, W.: Arch. f. exper. Zellforsch. **5**:269, 1928. (*b*) Moen, J. K.: J. Exper. Med. **6**:247, 1935. Reeves, D. L.: Bull. Johns Hopkins Hosp. **55**:245, 1934.

161. Seemann, G.: Verhandl. d. deutsch. path. Gesellsch. **25**:77, 1930; Beitr. z. path. Anat. u. z. allg. Path. **85**:303, 1930.

addition, though denied in the past, Aschoff [162] and Herzog [106] have conceded a similar conversion in the case of monocytes. Therefore, within the past few years, the number of strongest opponents of the theory of the transformation of polyblasts has become smaller. It seems noteworthy that Herzog, as a pupil of Marchand, admitted that the lymphocytes have at least the relative potency (*relative Potenz*) of a conversion into polyblasts, just what Maximow emphasized in 1902. Even so, the fact that every lymphocyte becomes a polyblast under inflammatory conditions has never been pointed out.

Mallory [163] in 1898 emphasized the hematopoietic function of the vascular endothelium, but Maximow claimed that hematopoiesis of the endothelium does not occur in adults, though he demonstrated it in early embryonic stages. Later the association of the adventitial cells (histiocytes) and their close connection with the endothelium were announced by Schridde and Herzog and subsequently substantiated by Marchand [164] (1924). Accordingly the histiocytes and the small lymphocytes produced by the endothelium would be of endothelial genesis. Maximow [19b] and I [165] failed to note evidence of hematopoietic functions in cultivated vascular endothelium. Histiocytes are not derived from endothelium by dissolution [106] or conversion; they differ morphologically and functionally from ordinary vascular endothelium; the latter plays no part in vital storage. The newly growing endothelial cells resemble fibrocytes in every respect, growing continuously as do vessels. These cells therefore represent a separate entity.

Hueper and Russell [166] reported a transformation of monocytes into endothelial-like cells, which took capillary form. Before their conclusions can be accepted a conversion into fibrocytes must be excluded. Cultures of embryonic endothelium did not reveal hematopoietic potentialities.[165]

Histogenous Reaction.—Similar to the condition in the body, mesenchyme in vitro has demonstrated the existence of three different varieties of connective tissue cells:

1. Mature fibrocytes producing fibers show abundant growth and are easily cultivated. Their differentiation into phagocytes is still debated. Under ordinary circumstances they behave in a uniform fashion different from that of phagocytes. Inflammation even of embryonic con-

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162. Karmally, A.: Beitr. z. path. Anat. u. z. allg. Path. **82**:92, 1929.

163. Mallory, F. B.: J. Exper. Med. **3**:611, 1898.

164. Marchand, F., in Krehl, L., and Marchand, F.: Handbuch der allgemeinen Pathologie, Leipzig, S. Hirzel, 1924, vol. 4, pt. 1, p. 1.

165. Silberberg, M.: Arch. f. exper. Zellforsch. **9**:36, 1929; Verhandl. d. deutsch. path. Gesellsch. **25**:144, 1930.

166. Hueper, W. C., and Russell, M. A.: Arch. f. exper. Zellforsch. **12**:407, 1932.

nective tissue produced in vitro by toxic, bacterial or chemical agents failed to prove Grawitz'[167] and von Möllendorff's[168] contention that there is hematopoiesis.[169] As to their conversion into true phagocytes no definite evidence has been detected. During the first few hours after explantation fibroblasts of the connective tissue may assume the appearance of histiocytes, but this does not prove that the changed cells have really become converted into functional macrophages, though one cannot deny the possibility.[170] Experiments in proof of such a transformation cannot withstand all criticisms. The term fibroblast should be discarded, as it may be misleading. Different varieties of cells show a similar appearance and have been grouped together by some authors as fibroblasts.

2. Undifferentiated mesenchymal cells have been described by Maximow[19b] and retain cytopoietic faculties during life. As long as they are not excluded as a possible source for the production of the so-called transitional cells (fibroblasts), one is not justified in generalizing and attributing such a possibility to the fibrocytes in the strict sense.

3. Histiocytes (resting wandering cells, clasmatocytes, rhagiocrin cells, leukocytoids, lymphocytoids, adventitial cells, pericytes) are the typical macrophages with the ability of further development into fibrocytes. Other cytopoietic functions[164] could not be established.

In cultures of mesenchyme of 2 to 3 day chick embryos I[171] have observed the commencing differentiation into fibrocytes and histiocytes long before blood cells, in the strict sense, were seen. This observation has been supported by Mihálik[49] in cultures of the brain of 3 day chick embryos.

Alveolar phagocytes are morphologically and functionally identical with histiocytes in every respect. In cultures of lung tissue no alveolar epithelial lining was discovered. On the other hand, in the interalveolar connective tissue Maximow, Lang[19a] and others demonstrated the existence of typical histiocytes, so-called septum cells. Henke and I,[172] in investigations on the origin and the nature of the large exudation cells in the lungs, did not detect participation of vascular endothelium

167. Grawitz, P.: Virchows Arch. f. path. Anat. **232**:35, 1920.

168. von Möllendorff, W.: Ztschr. f. Zellforsch. u. mikr. Anat. **12**:274, 1930; **12**:559, 1931.

169. Silberberg, M.: Virchows Arch. f. path. Anat. **270**:667, 1929. Silberberg, M., and Orzechowski, G.: ibid. **269**:289, 1928. Tannenberg, J.: Arch. f. exper. Zellforsch. **11**:165, 1931. Rix, E., and Greifenstein, A.: Sitzungsb. d. Gesellsch. z. Beförd. d. ges. Naturw. zu Marburg **64**:49, 1930.

170. Bloom, W.: Arch. f. exper. Zellforsch. **11**:145, 1931.

171. Silberberg, M.: Verhandl. d. deutsch. path. Gesellsch. **23**:456, 1928.

172. Henke, F., and Silberberg, M.: Verhandl. d. deutsch. path. Gesellsch. **24**: 119, 1929; **26**:114, 1931.

or the presence of alveolar epithelium. Nonspecific and tuberculous irritation in vitro revealed that the ameboid septum cells migrate into the alveoli and act as macrophages (alveolar phagocytes, cardiac failure cells). Later these cells may be converted into fibrocytes. Thus there is no evidence in their behavior indicative of an epithelial origin.

Explants of spleen and bone marrow show that the specificity of their cells is preserved during the first days of cultivation, but later only pure cultures of fibrocytes, without the slightest sign of their origin, result. Thus all varieties of cells must have undergone changes in the same direction. Such strains of so-called fibroblasts were kept alive by Carrel for over twenty years.

The question arises as to whether such processes would mean a dedifferentiation.[173] As epithelium likewise can be cultivated indefinitely and yet retain its specificity, conclusions as to dedifferentiation cannot be drawn. Fischer [1d] stated the opinion that dedifferentiation occurs only if proliferation is overaccelerated. On the other hand, transplants of hematopoietic tissue show a similar behavior so far as the various cell elements undergo differentiation, finally resulting in their transformation into fibrocytes, an indication that, in spite of the variety of the cells in the tissue, the tissue differential [174] is less marked.[175] Growth in vitro therefore would mean not dedifferentiation but differentiation in one specific direction.

With reference to hematology, the assumption of an omnipotent cell and also the unitarian theory obtain new support. Chlopin described erythropoiesis from undifferentiated cells in cultured connective tissue of the axolotl. As regards the monocytes, no agreement has been reached so far: Some investigators have proved their origin from histiocytes and others from lymphocytes. From my investigations,[176] I favor the former view, but I am unable to exclude the possibility of the latter hypothesis. According to Maximow [19b] and Bloom,[177] monocytes should not be considered an entity but rather should be considered a conglomeration of various cell types. On the other hand, the monocytes are equipped with particular reactivity under pathologic conditions, such as malaria, angina, some tropical diseases and leukemia and under the influence of Bacterium monocytogenes and other organisms. Therefore, from the pathologic angle one is justified in postulating a specific third group of cells ranking equally with the lymphocytes and

173. Champy, C.: Compt. rend. Soc. de biol. **77**:282, 1914.
174. Loeb, L.: Physiol. Rev. **10**:342, 1929.
175. Silberberg, M.: Arch. Path. **20**:216, 1935.
176. Silberberg, M.: Virchows Arch. f. path. Anat. **267**:483, 1928.
177. Bloom, W.: Proc. Soc. Exper. Biol. & Med. **24**:567, 1927; footnote 160.

leukocytes. A modified and limited unitaristic theory in accordance with these principles is acceptable.[178]

Serosal cells have been separated as being different from other cells.[179] Explants of peritoneal, mesenteric and omental exudates revealed a membranous epithelial-like growth of the serosal cells, which do not become converted into histiocytes and which take no part in vital storage. At later stages they may assume the appearances of fibrocytes, or desmocytes.[180] Mesothelium seems to link the epithelial and connective tissues. These observations in vitro corroborate the histogenesis of endotheliomas of the serous membranes and their position within the oncologic system.

PRODUCTION OF FIBERS

In fibrin, absolutely cell free, Chlopin [181] did not observe the formation of fibers or substances indicative of their production, and he concluded that only living cells can produce fibers, in opposition to the opinion of a minority of other observers. Bloom [182] reported the formation of elastic fibers in cultures of embryonic heart and aorta.

According to Maximow,[183] argentophilic or reticulum fibers are first produced extracellularly but later lose their silver impregnation, forming large bundles of collagenous fibers. Levi [184] stated that both intracellular and extracellular formation are possible. Cell growth occurs previous to the formation of fibers.[185] Chemical factors, in addition, play an important rôle in the production of fibers. Odieffs [186] recently noted differences in their production under the influence of various amino-acids.

SPECIFIC INFLAMMATION

Explanted lung and lymphoid tissue was inoculated with Bacillus Calmette-Guérin.[187] Infection with B C G did not reveal toxic activity

178. Silberberg, M.: Virchows Arch. f. path. Anat. **274**:820, 1930; Verhandl. d. deutsch. path. Gesellsch. **26**:110, 1931; Klin. Wchnschr. **9**:174, 1930. Henke, F., and Silberberg, M.: ibid. **11**:49, 1932.

179. Maximow, A.: Arch. f. exper. Zellforsch. **4**:1, 1927.

180. Schopper, W.: Beitr. z. path. Anat. u. z. allg. Path. **88**:451, 1932.

181. Chlopin, N.: Virchows Arch. f. path. Anat **252**:25, 1925; Ztschr. f. Zellforsch. u. mikr. Anat. **2**:324, 1925; Arch. f. exper. Zellforsch. **12**:11, 1931.

182. Bloom, W.: Arch. f. exper. Zellforsch. **9**:6, 1929.

183. Maximow, A.: Zentralbl. f. allg. Path. u. path. Anat. **43**:145, 1928.

184. Levi, G.: Arch. f. exper. Zellforsch. **11**:178, 1931.

185. Wjereszinsky, A.: Folia haemat. **5**:41, 1924. Bloom.[182]

186. Odieffs, D.: Compt. rend. Soc. de biol. **110**:940, 1932.

187. (*a*) Maximow, A.: Ann. Inst. Pasteur **42**:225, 1928. (*b*) McKinney, R.: Arch. f. exper. Zellforsch. **9**:14, 1930. (*c*) Lang, F. J.: J. Infect. Dis. **37**:430, 1925. (*d*) Maximow, A.: ibid. **34**:549, 1924; **37**:418, 1925. (*e*) Lewis, W. H.: Am. Rev. Tuberc. **15**:616, 1927.

on cellular elements of rabbit tissue.[187e] The epithelioid cells are hypertrophic macrophages;[188] their formation is due to a differentiation of the indifferent interalveolar reticulum, and later they may correspond to histiocytes. Necrosis similar to caseation and the formation of multinucleated giant cells have been reported. Bovine bacilli inhibit the growth of tissue and diminish the inflammatory reaction. Thus a very specific reaction is obtained: After eleven days of cultivation all characteristic tuberculous lesions are found—necrosis, epithelioid cells and giant cells, provided no subcultures are made. Transformation of histiocytes into epithelioid cells usually does not occur. The origin of giant cells by fusion of emigrated septum cells or by their proliferation or by fusion of polyblasts has been proved. I have repeatedly observed the formation of multinucleated giant cells as early as within four days. The results of tissue culture support the view that giant cells are not of specific significance, as they may be produced under varying conditions. Epithelioid cells, polyblasts and giant cells all show phagocytosis of the bacilli, which multiply within the cultivated cells.

Timofejewsky and Benewolenskaja,[189] by infecting the buffy coat with tubercle bacilli, demonstrated a development of lymphocytes into epithelioid and giant cells. In infected explants of leukemic blood a more pronounced resistance of the immature myeloid cells to the bacilli was noticeable, since such cells do not participate in phagocytosis. By infecting embryonic and adult parenchymatous tissue they discerned that every reaction depends on the resistance of the bacilli; if the bacilli are highly virulent, necrosis of the parenchyma and rarely giant cells are seen. Bacilli of weak virulence provide better conditions for the reaction of the mesenchyme in the formation of epithelioid and giant cells.

Two observations of sympathetic tuberculous ophthalmia are recorded.[190]

Cultivation of other specific granulomas revealed similar results: Pinus[191] infected the spleen of the white mouse with Bacillus mallei and saw a marked growth of macrophages, with phagocytosis of the bacilli.

In a study of leprosy Salle[192] cultivated connective tissue elements containing acid-fast organisms. Benewolenskaja[193] investigated the

188. Wermel, E.: Virchows Arch. f. path. Anat. **281**:297, 1931.

189. Timofejewsky, A. D., and Benewolenskaja, S.: Arch. f. exper. Zellforsch. **6**:230, 1926.

190. Sellmann, L.: Ztschr. f. Augenh. **83**:168, 1934. Purtscher, A.: ibid. **83**: 163, 1934.

191. Pinus, A.: Arch. f. exper. Zellforsch. **10**:109, 1930.

192. Salle, A. J.: Infect. Dis. **54**:343, 1934.

193. Benewolenskaja, S. W.: Arch. f. exper. Zellforsch. **13**:37, 1932.

reaction of embryonic tissue and of human leukocytes to leprosy bacilli and observed the formation of epithelioid cells and phagocytes, as in tuberculosis.

With Bacillus typhosus Lewis [194] reported a rapid formation of vacuoles in the macrophages of the intestine of chick embryos.

IMMUNOLOGY

Lambert and Hanes [195] first reported a relatively slight sensitiveness of tumor and normal splenic cells to the toxic action of heterologous serum. Cultures of fibroblasts may remain active in such serum for a certain length of time, but the injurious effect becomes less manifest in the case of tumor cells.[1d] Loeb's [196] recent contributions are very conclusive: By cultivating thyroid gland tissue of the guinea-pig in autogenous or homogenous serum or plasma and by counting the mitotic figures in the acinar epithelium, he found no special differences due to homoiotoxins. Similarly the differences between the effects of homogeneous and heterogeneous plasma on growing tissue are much less than in the corresponding experiments carried out in transplantation. Conditions in vitro are obviously different from those within the body, not only on account of the absence of host tissue but also in regard to the quantity of blood plasma present; therefore the amount of the homotoxins or heterotoxins available is soon exhausted.

In tissue culture, though any essential activity of the blood or any influence of the nervous system is absent, antibodies and an allergic reaction have been observed. Sereni and Garofolini [197] sensitized spleen and bone marrow to horse serum and cultivated the cells in mediums to which minute amounts of antigen (1:10,000 to 1:20,000) were added; they observed anaphylactic shock, shown by extensive cellular degeneration. The hyperallergy, however, was obtained only up to seven days (third passage) in a normal medium. The formation of anaphylactic antibodies seemed exclusively bound up with the hematopoietic organs. Serum from rabbits which had received injections of pulp of embryonic chick heart showed definite cytotoxic action on explanted fibroblasts and epithelial cells, the degree of the inhibition of growth depending on the concentration of the serum used.[198] Several investigators demonstrated the production of bactericidal substances, e. g., Komatsu [199] in cultures of the spleen and bone marrow of a

194. Lewis, M. R.: J. Exper. Med. **31**:293, 1920; Anat. Rec. **18**:239, 1920.
195. Lambert, R. A., and Hanes, F. M.: J. Exper. Med. **14**:129, 1911.
196. Loeb, L.: Physiol. Rev. **10**:547, 1930.
197. Sereni, E., and Garofolini, L.: Arch. f. exper. Zellforsch. **13**:53, 1932.
198. Fellinger, K.: Arch. f. Exper. Zellforsch. **13**:310, 1932. Verne, J., and Oberling, C.: Compt. rend. Soc. de biol. **109**:860, 1932.
199. Komatsu, S.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **71**:76, 1931.

rabbit infected by B. typhosus. On the other hand, introduction of the antigen to the cultures was not followed by a development of bactericidal properties. Some immunization has been shown with various toxins,[200] tetanus,[201] diphtheria,[202] anaerobes (Bacillus novyi, Bacillus oedematiens),[203] scarlet fever [204] and staphylococci.[205]

Rich and Lewis [206] observed that washed cells of tuberculous allergic bodies retain their hypersensitivity to tuberculin. Therefore the reaction is not necessarily due to the existence of free circulating antibodies but is based on the action of the protein of the bacilli on the cells, independent of nervous and other mechanisms. Aronson [207] likewise noticed inhibition of migration and growth of explanted tuberculous tissue under the influence of tuberculin but not by the use of horse serum, as reported by other observers. There is no obvious reason why the mechanism underlying the tuberculous reaction should be different from that of anaphylaxis and the Arthus phenomenon. Spassky [208] noted a growth-inhibiting and cytotoxic effect when splenic or chick embryonic serum was used. Hueper and Russell [209] discerned that antileukocytic serum has an antiproliferative and cytolethal effect on leukocytes and other tissue cells of the same species against which the serum has been prepared. Serum of leukemic chickens was ineffective against leukemic cells from human beings. In a few cases favorable therapeutic results have been obtained by the use of an antileukemic serum.[209]

VIRUS

As the existence of a virus is bound up with the living cell, investigations in this field hold much promise. Many viruses can now be grown.

Parker and Nye [210] and Carrel and Rivers [211] inoculated explanted chick embryo tissue in flask cultures with from 25 to 250 units of

200. Meier, R.: Arch. f. exper. Zellforsch. **15**:67, 1934.
201. Toyoda, M.: Arch. f. exper. Zellforsch. **10**:463, 1931.
202. Krontowski, A. A., and Jazimirska-Krontowska, M.: Microbiol. J. **9**:205, 1929.
203. Barg, G. S.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **79**:187, 1933.
204. Spassky, N. N.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **81**:336, 1934.
205. Kato, M., and Muroo, S.: Mitt. a. d. med. Akad. zu Kioto **12**:1380, 1934.
206. Rich, A. R., and Lewis, M. R.: Bull. Johns Hopkins Hosp. **50**:115, 1932.
207. Aronson, J. D.: J. Immunol. **25**:1, 1933.
208. Spassky, N. N.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:265, 1932.
209. Hueper, W. C., and Russell, M. A.: Arch. Path. **13**:584, 1932.
210. Parker, F., and Nye, R. N.: Am. J. Path. **1**:325 and 337, 1925.
211. Carrel, A., and Rivers, T. M.: Compt. rend. Soc. de biol. **96**:848, 1927.

smallpox virus. In eight days the units had increased four hundredfold. These figures show that an explanted chick embryo can produce as much virus as a calf. Rivers [212] has successfully used such lymph for the vaccination of babies. Ch'en [213] investigated variations in the potency of the virus of vaccinia, which can multiply and increase only in the presence of living cells for an unlimited time. Vaccinia "granules" fail to remain potent if cultured with dying cells, thereby showing the same behavior as the entire vaccine.[214] The potency of the granules increases with the age of the cultures, but the filtrate is ineffective.

Andrewes [215] cultivated the virus of herpes; Dochez, Mills and Kneeland,[216] that of coryza; Hoshizaki,[217] that of Manchurian typhus, and Haagen and Theiler,[218] that of yellow fever. The result of all such work has unvariably been the same, namely, multiplication.

The viruses of Rickettsia, supposedly being filtrable, were the target for systematic investigations: [219] Rickettsia prowazeki, of typhus, or spotted fever, Rickettsia orientalis, of tsutsugamushi disease,[220] and Rickettsia dermacentroxenus [221] all were analyzed. Pinkerton and Hass [222] have shown that optimum growth occurs at 32 C., when the organisms increase in number rapidly within the cytoplasm of infected cells, distended with the organisms taken from exudate in the scrotum up to fourteen days. Normal tissue does not become infected in vitro, even though in close proximity to heavily infected cells. A rise in temperature has a detrimental effect on the growth of the organisms, probably owing to an increase of the defensive mechanism within the cells.

In the majority of cases the viruses were cultured in chick embryo mediums at 39 C. Yen and Chung [223] observed multiplication of Leish-

212. Rivers, T. M.: J. Exper. Med. **54**:453, 1931.

213. Ch'en, W. K.: Proc. Soc. Exper. Biol. & Med. **31**:1252, 1934.

214. Craciun, E. C., and Oppenheimer, E. H.: J. Exper. Med. **43**:815, 1926.

215. Andrewes, C. H.: J. Path. & Bact. **33**:301, 1930.

216. Dochez, A. R.; Mills, K. C., and Kneeland, Y., Jr.: Proc. Soc. Exper. Biol. & Med. **28**:513, 1931.

217. Hoshizaki, S.: Kitasato Arch. Exper. Med. **9**:155, 1932.

218. Haagen, E., and Theiler, M.: Proc. Soc. Exper. Biol. & Med. **29**:435, 1932.

219. Nigg, C., and Landsteiner, K.: Proc. Soc. Exper. Biol. & Med. **28**:3, 1930. Suzuki, S.: Arch. f. exper. Zellforsch. **15**:440, 1934.

220. Nagayo, M., and others: Jap. J. Exper. Med. **9**:87, 1931. Nishibe, M.; Hosono, S., and Miyazawa, M.: Arch. f. exper. Zellforsch. **13**:465, 1932.

221. Wolbach, S. B., and Schlesinger, M. J.: J. M. Research **44**:231, 1923.

222. Pinkerton, H., and Hass, G. M.: J. Exper. Med. **54**:307, 1931; **56**:131, 1932.

223. Yen, A. C. H., and Chung, H. L.: Proc. Soc. Exper. Biol. & Med. **31**: 1258, 1934.

mani Donovani. Similar results were observed with the viruses of psittacosis,[224] avian pest, or Newcastle disease,[225] foot and mouth disease,[226] hog cholera [227] and pseudorabies,[228] if explanted along with testicular tissue of an immunized guinea-pig.

Viruses causing disease in rabbits also have been cultivated, namely, the viruses of myxomatosis,[229] fibromatosis,[230] aseptic orchitis [231] and parotitis.[232] Rabbit virus III [233] still proved infectious in the high dilution of 1 : 300,000,000. The virus taken from tissue cultures was more virulent than that from the animal itself, as judged by the severity of lesions when susceptible animals were inoculated. Passage of the cultured virus through animals reduced its potency. Even the fluid expressed from the plasma clot of the culture was virulent. The interstitial cells of explanted rabbit testicle were susceptible to the virus, but not to the sex or primary cells. The cells containing the inclusions continued to multiply until overcome by the virus, which finally destroyed them.

Trager [234] grew the virus of grasserie in silkworms.

The work of Krontowski [235] was concerned with the practical cultivation of dermovaccines and neurovaccines, and the results were in accordance with those just mentioned.

Otto [236] reproduced bacteriophages by the addition of methyl violet, snake poison and mercury to the cultures.

The problems concerning the virus of the Rous sarcoma will be discussed in the following section, on tumors.

TUMORS

Corresponding to the behavior of epithelium and connective tissue in general, successful cultivation of sarcomatous material is distinctly easier than that of material from epithelial tumors. Spontaneous sar-

224. Bland, J. O. W., and Canti, R. G.: J. Path. & Bact. **40**:231, 1935.
225. Topacio, T.: Philippine J. Sc. **53**:245, 1934.
226. Maitland, M. C., and Maitland, H. B.: J. Comp. Path. & Therap. **44**:106, 1931.
227. Hecke, F.: Zentralbl. f. Bakt. (Abt. 1) **126**:517, 1932.
228. Traub, E.: J. Exper. Med. **61**:833, 1935.
229. Benjamin, B., and Rivers, T. M.: Proc. Soc. Exper. Biol. & Med. **28**: 791, 1931.
230. Faulkner, G. H., and Andrewes, C. H.: Brit. J. Exper. Path. **16**:271, 1931.
231. Doerr, R.: Tijdschr. v. hyg. Mikrobiol. u. Serol., 1926.
232. Andrewes, C. H.: Brit. J. Exper. Path. **11**:23, 1930.
233. Topacio, T., and Hyde, R. R.: Am. J. Hyg. **15**:99, 1932.
234. Trager, W.: J. Exper. Med. **61**:501, 1935.
235. Krontowski, A. A., and others: Compt. rend. Soc. de biol. **114**:424, 1933.
236. Otto, R.: Ztschr. f. ärztl. Fortbild. **20**:253, 1923.

comas of animals, chicken sarcoma and Crocker's and Jensen's rat sarcoma are suitable tumors, allowing explantation and transplantation without difficulty.

Growth.—Explanted sarcomas show migration and growth of two main cell types: (1) ameboid macrophages and (2) pure strains of "fibroblasts."

According to Carrel and Ebeling,[237] the malignant fibroblasts of rat sarcoma possess the morphologic characters and the mode of locomotion of normal rat fibroblasts, but their colonies are larger and their cells are coarser and more loosely arranged.[238] They do not show abnormalities or degeneration. Slight physiologic differences are reported: sarcomatous cells, unlike normal cells, liquefy a coagulum of rat plasma; digested calf's liver promotes an unlimited proliferation of sarcomatous cells but fails to support the growth of normal fibroblasts.

Differences as to the malignant principle within the two cell types are seen: Reimplantation of tumor fibroblasts, cultivated for three or four days previous to inoculation, gave a diminished malignant growth; when cultured for eight days, no malignant growth developed. On the other hand, after from ten to thirty days in vitro malignant properties developed in the reinoculated "monocytoid-like macrophages." As the presence of these macrophages is essential for the preservation of malignant growth, certain undifferentiated cells of the mesenchyme are probably the bearers of the as yet unknown malignant principle. Thus, pure cell strains of the macrophages isolated from sarcomas showed the same grade of malignancy as the original material after sixteen months of cultivation.[237] Hirschfeld and Klee-Rawidowicz [238] observed good growth and a marked increase in the number of macrophages in the tissue of a Jensen sarcoma explanted in heterologous plasma.

Lambert [239] reported an increase in the number of chromosomes within sarcoma cells. Lewis and Lockwood [240] noted eighty-four chromosomes, all tetraploid, in sarcoma cells instead of forty-two as in normal cells but no multipolar or asymmetric mitosis. These observations were supported by Carrel and Ebeling.[237] Hirschfeld [238] observed subdiploid, diploid and polyploid chromosomes and pluripolar asymmetric mitosis. These differences cannot be employed in the differentiation

237. Carrel, A., and Ebeling, A. H.: J. Exper. Med. **43**:461, 1926; **48**:105 and 285, 1928; Arch. f. exper. Zellforsch. **5**:125, 1927; Compt. rend. Soc. de biol. **91**: 1067, 1925; **93**:1083, 1925; **97**:19, 1927.

238. Hirschfeld, H., and Klee-Rawidowicz, E.: Ztschr. f. Krebsforsch. **30**: 406, 1929.

239. Lambert, R. H.: J. Exper. Med. **17**:499, 1913.

240. Lewis, M. R., and Lockwood, J.: Bull. Johns Hopkins Hosp. **44**:187, 1927.

between sarcoma and normal cells. Lewis and Gey [241] have stated that the presence of an abundant number of mitrochondria is characteristic of sarcoma cells.

These observations in tissue culture prove that the principle of the malignant process is endogenous and situated within the tumor cells. However, a few isolated publications have appeared concerning the reproduction of sarcomas in vitro by exogenous irritation. Fischer and Laser,[242] by the addition of arsenic to explanted chicken spleen, observed a transformation of normal into sarcoma cells, as proved by reimplantation of the cultured material into animals. Laser [243] recorded a similar result after irritating growing mesenchyme with tar. On account of the importance of such results, further experiments are required to determine whether one is dealing with special circumstances or whether the same results can be successfully obtained on a larger scale.

Special attention has been given to the Rous sarcoma. Fischer [112] emphasized its unlimited power of cultivation in association with embryonic chicken tissue. Reimplantation into animals reveals that the original malignant growth tendency is still present. In spite of all objections, the fact remains that the malignant growth principle of the Rous sarcoma, whether of a cellular nature or of a virus-like type, can be stimulated symbiotically with normal tissues.

As to the cultivation of gliomas, the first attempt showed that of three astrocytomas and one spongioblastoma multiforme only the latter grew.[72a] Buckley [72b] succeeded in five of ten cases of spongioblastoma. He confirmed the polymorphism of the glia cells; he observed a growth of spindle cells of spongioblastic type and cells of stellate or astrocytic form. Russell and Bland [72c] explanted twenty gliomas, including medulloblastomas and astrocytomas. They reported the migration of tumor cells exhibiting great plasticity of form but retaining their morphologic differentiated characters, thus proving a preservation of the cell specificity. Therefore, the classification of tumors according to the various cells is justified. Actual multiplication of cells was seen in two cases of spongioblastoma multiforme. Growth of ependymomas and papillomas of the choroid plexus did not occur.

As cancer cells have a tendency to invade normal tissue and to destroy it, Fischer [1d] suggested explanting normal tissue simultaneously with tumor material. Using this method, he prevented liquefaction of the plasma and succeeded in cultivating cancer cells for an unlimited

241. Lewis, W. H., and Gey, G.: Bull. Johns Hopkins Hosp. **34**:369, 1923; Arch. f. exper. Zellforsch. **5**:143, 1928.

242. Fischer, A., and Laser, H.: Arch. f. exper. Zellforsch. **3**:363, 1927.

243. Laser, H.: Arch. f. exper. Zellforsch. **6**:142, 1928.

length of time. Others failed to support these findings with culture of carcinomas of the human breast and gallbladder.[244] On the whole, there seems no doubt that animal cancers are more easily explantable if associated with normal tissue. The cancer cells reveal their destructive growth activity against themselves and normal cells; they regenerate and multiply under the influence of irritation, the necrosis of their own cells acting as a growth-promoting factor. If any stroma is present, the cancer cells stimulate its growth. One has apparently to deal with the same phenomenon of symbiosis as discussed under sarcomatous growth. Erdmann,[245] in earlier investigations, stated the conclusion that cancer cells should remain alive only if explanted with stroma. According to more recent studies, pure cultures of cancer cells may retain their malignant tendency for years. Cancer cells do not grow more rapidly than normal regenerating epithelium, but they overgrow the epithelium and in the end destroy it. The morphologic behavior of normal cultivated epithelium resembles that of atypical cancer cells. In explants of normal mammary gland Maximow [24] demonstrated the formation of atypical epithelial cells, just as in cancerous tissue. This was more pronounced under the influence of bone marrow extract than under that of embryonic juice. Kapel [48] simultaneously cultured pure epithelial cells with fibroblasts and observed in the second transfer that the fibroblasts were surrounded by epithelium and were dead. If after the third passage death of the fibroblasts had occurred from proteolytic fermentation, atypical growth of the epithelium was noticed, occasionally revealing an infiltrative character. These observations are important with reference to the conversion of normal epithelial cells into malignant cells. Though so far normal epithelial cells have not been converted into true cancer cells, these and similar observations indicate that it may be possible in the future to reproduce such a transformation experimentally in cultures.

The various strains of cancer show slight differences with respect to fibrinolysis: mouse and rat plasmas are more easily liquefied than chicken plasma; here again one must distinguish slight differences as to the behavior of spontaneous mammary tumors and Ehrlich's mouse carcinoma.[246] The cancerous cells grow in a membranous-like manner, the number of mitotic figures being the same and chromosomes being less numerous than normally.

Metabolism.—Naturally, the metabolism of tumor cells was particularly studied. Loeb [247] pointed out that the injurious action exerted by tumor cells is probably due to excreted abnormal products of metab-

244. Lauche, A.: Verhandl. d. deutsch. path. Gesellsch. **25**:296, 1930.
245. Erdmann, R.: Zentralbl. f. Bakt. (Abt. 1) **93**:194, 1924.
246. Santesson, L.: J. Exper. Med. **55**:281, 1932; **56**:893, 1932.
247. Loeb, L.: Ann. Int. Med. **4**:669, 1931.

olism of tumor cells or to abnormal enzymes. Such an abnormal metabolism has been discerned by Warburg. In tumor cells, as in yeast cells, the enzymatic splitting of carbohydrates and the production of lactic acid by these means is very marked, not only under aerobic but under anaerobic conditions. Although these findings are very interesting and important, they still do not differentiate carcinomatous from normal tissues; particularly regenerating tissues approach the metabolism of cancer cells more closely than does embryonic tissue.

Demuth and Riesen [248] found that even fibrinolysis may not be specific of tumor growth. Krontowski and his co-workers,[249] after the treatment of tumor particles with mono-bromacetic and mono-iodacetic acid and reimplantation into rats drew the following conclusions: Sarcomatous tissue is more susceptible to inhibition by acids than normal embryonic connective tissue; no growth takes place in vitro or in vivo if glycolysis stops or is retarded. Sarcoma cells turn phenol red a golden yellow, whereas normal cells turn it pinkish orange, thus indicating a higher glycolytic rate.[237] Chambers and Ludford [250] found the intracellular hydrogen ion concentration of mouse tumors to be p_H 6.8 $\pm$ 0.1, a value comparable to that of normal cells; after injury or cytolysis of both cancerous and normal cells a decrease was noticed to p_H 5.6 or less. The intracellular p_H is figured at 7.2; it is not changed under any circumstances.

Sarcomatous and cancerous tissues and cells show the same biologic behavior as to glycolysis. Tissue cultures revealed that tumor cells do not grow better than normal cells under anaerobic conditions but are more sensitive and therefore less resistant.

In summarizing, one may state that between tumor and normal cells only quantitative differences exist; conclusions as to any differences in their biologic principles are not justified at present.

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248. Demuth, F., and von Riesen, cited by Doljansky.[116]

249. Krontowski, A. A., and others: Arch. f. exper. Zellforsch. **3**:32, 1927; **5**:117, 1928; **11**:93, 1931.

250. Chambers, R., and Ludford, R. J.: Proc. Roy. Soc., London, s. B **110**: 120, 1932.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Martin Silberberg, research assistant in pathology in Dalhousie University, Halifax, Canada, has been appointed professor of pathology in the University of Panama City and director of the department of pathology in Santo Tomas Hospital, Panama, P. R.

Ronald G. Canti, clinical pathologist at St. Bartholomew's Hospital, London, best known for his sensational cinematographic record of events in tissue culture and for his Canti cancer film, died on Jan. 7, 1936, at 53 years of age.

Edgar Sydenstricker, scientific director of the Milbank Fund and for many years chief statistician of the United States Public Health Service, died on March 19, 1936, aged 54 years.

William H. Bergtold, formerly professor of pathology in the University of Buffalo and in the University of Denver and also known as an ornithologist, died on March 19, 1936, at the age of 70 years.

Ettore Marchiafava, professor of morbid anatomy (1883-1916) and then of clinical medicine (1916-1921) in the University of Rome, one of the great Italian malariologists, died on Oct. 22, 1935, aged 88 years.

Roger Perkins, professor of hygiene and bacteriology from 1911 to 1930 in Western Reserve University, Cleveland, and previously member of the department of pathology, has died at the age of 62 years. He was connected with the health department of Cleveland for many years.

Captain S. R. Douglas, pioneer in clinical bacteriology, died on Jan. 20, 1936, at the age of 65 years. Captain Douglas was invalided from the Indian Medical Service in 1901. He worked with Sir A. E. Wright at St. Mary's Hospital, London, on the opsonic index and vaccine therapy from 1904-1914. In 1918 he became director of the department of experimental medicine in the laboratories of the Medical Research Council at Hampstead, where he directed important researches on virus diseases and other problems.

Rudolf Maresch, director of the Pathologic-Anatomic Institute of the University of Vienna since 1923, has died at the age of 68 years.

Paul Dittrich, from 1893 to 1929 professor and head of the medicolegal institute in Prague, died last January in his seventy-seventh year.

The Guggenheim Foundation has announced the appointment to fellowship of John T. Culbertson, instructor in bacteriology, Columbia University, for study of immunity against protozoan and helminthic diseases in man; the reappointment of Michael Heidelberger, chemist to the Presbyterian Hospital, New York, for study of the mechanism of immune reactions, and the reappointment of Morris Moore, research Fellow, the Barnard Free Skin and Cancer Hospital, St. Louis, for a comparative study of pathogenic fungi of North and South America.

Society News.—The Fourth International Congress for Experimental Cytology will meet at Copenhagen on Aug. 10 to 15, 1936. It is proposed to consider the following subjects: the physical chemistry of the cell, the histochemical problems of cell metabolism, experimental morphology, the electrophysiology of the cell, experimental cell pathology and the biology of irradiation. The secretary is Dr. Harald Okkels, Institute for Pathological Anatomy, Copenhagen, Denmark.

The Second International Congress of the Scientific and Social Campaign Against Cancer will be held in Brussels on Sept. 20 to 26, 1936. The address of the general secretariat is 13 rue de la Presse, Brussels, Belgium.

The eleventh congress of the International Society of the History of Medicine will be held in September 1938 at Athens, Istanbul, Sophia and Zagreb. The chief subjects for discussion will be the religious origins of hippocratic medicine, the hippocratic idea in modern medicine and medical folk-lore.

The sixty-fifth annual meeting of the American Public Health Association will be held in New Orleans on Oct. 20 to 23, 1936.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Chemistry

Hematopoiesis and Blood Vessel Formation in Bone Marrow. W. Gohs, Virchow's Arch. f. path. Anat. **294**:103, 1934.

Gohs reviews the controversy as to whether erythropoiesis is an extravascular process, as maintained by Maximow and others, or an intravascular one, as held by Sabin. From his own studies on chickens he concludes that the process of erythropoiesis and vessel formation is the same in the bone marrow as in the yolk-sac and liver of the embryo chick. The marrow has two sets of capillaries. One, termed arterial, originates from the arteries and supplies the marrow with plasma. In the marrow, groups of erythroblastic cells become surrounded by a layer of reticulo-endothelium. The erythroblasts attract from the tissue a fluid that has come from the arterial capillaries. Thus is formed a system of venous capillaries which joins the veins but has no connection with the arterial capillary system. Erythropoiesis takes place in the venous capillaries. In hyperplastic states of the marrow erythropoiesis occurs also outside the vessels. Myelopoiesis is normally an extravascular process, the leukocytes wandering into the venous capillaries.

O. T. Schultz.

Experimental Hyperpigmentation Due to Female Sex Hormone. G. Guldberg, Virchows Arch. f. path. Anat. **294**:213, 1934.

In castrated male guinea-pigs given daily injections of crystalline female sex hormone (folliculin), there occurred marked pigmentation of the mammae, mammary hypertrophy with the secretion of a milky fluid, and slight pigmentation of the genitalia. Quantitatively and qualitatively the hyperpigmentation was identical with that which occurs in women during pregnancy. The degree of pigmentation bore a relation to the quantity of hormone injected. The pigmentation is due to the direct action of the hormone on the melanoblasts, which are activated and stimulated to increased function by the hormone. In nongravid women it was possible to bring about deep pigmentation of the nipples, chloasma, pigmentation of the upper lip and pigmentation of the linea alba by repeated injection of the hormone. The hormone caused only slight increase in pigmentation in uncastrated male guinea-pigs, probably because of the antagonistic action of the male hormone. The slight increase in pigmentation sometimes observed in women whose ovarian function has been lost before the menopause is held to be the result of aging and is similar to the process that occurs after the menopause.

O. T. Schultz.

Experimental Study of the Tuberculous Tissue Reaction. F. Roulet, Virchows Arch. f. path. Anat. **294**:262, 1934.

Dead tubercle bacilli introduced parenterally evoke the same histologically characteristic tissue reaction as living bacilli. This suggests that the specific reaction is the result, not of the life processes of the organism, but of its physicochemical constitution. If this is true, killed tubercle bacilli of varying degrees of virulence should lead to varying degrees of tissue reaction. Roulet injected suspensions of killed bacilli intracutaneously into rabbits and studied the lesions histologically. Bovine bacilli caused a more marked tissue reaction than human

bacilli, and bovine bacilli of high virulence, a greater reaction than those of low virulence. Nonpathogenic acid-fast bacilli in the killed state led to the same kind of reaction as killed tubercle bacilli, suggesting that the reaction is evoked by the composition of the bacilli. Fractionation of the various strains of organisms by the method of Anderson was undertaken, and the various products introduced intracutaneously. Only the phosphatide fraction led to the characteristic reaction. Phosphatides of other origin had no such effect, suggesting that the phosphatide of the tubercle bacillus is chemically specific or is contaminated by specific substances.

O. T. SCHULTZ.

REACTION OF THE EMBRYO TO CHEMICAL AGENTS. K. BAUER, Virchows Arch. f. path. Anat. **294**:476, 1935.

Sixty pages with thirty-four illustrations are devoted to the presentation of work done in Held's institute for anatomy at Leipzig on the tissue reactions of the embryo to certain chemical agents. Forming the basis of the investigation was the concept that multiplication of the cells of the tissues of the adult organism, such as occurs in regeneration, inflammation and neoplasia, is dependent on a return of differentiated cells to the embryonic state or that it results from the activity of previously slumbering and indifferent embryonic cells. How the cells of the embryo react to agents that set up inflammation in the adult organism has, however, received scant attention. Bauer used the chick embryo as the object of study. The agents used were benzene, a benzene-paraffin mixture, aniline, and aluminum powder. After a short period of incubation of the egg a small opening was made into the shell, the selected material was introduced, the opening sealed, and the incubation continued for from twenty-four to forty-eight hours. The embryos were then fixed and examined in serial sections. The localized granular degeneration and death of cells confirmed an older opinion that the embryo reacts to injurious agents only by degenerative processes and necrobiosis. But in portions of the embryo not killed by the chemical agents the ectoderm, entoderm and the supporting mesenchyme of the mesoderm were hypoplastic, supposedly as the result of an inhibition of growth by the chemical. The formation of blood vessels, and hematopoiesis, however, were not inhibited and were more active than normal. Bauer could find no support for Maximow's view that indifferent mesenchymal cells participate in the formation of blood vessels. He believes that blood vessels come only from vascular endothelium. He discusses the relation of differentiation and cell division in the light of his observations. Cell division is not a function of the nucleus alone but is influenced by the cytoplasm. This influence may be an important feature in the proliferation of tissue when the stimulus to division is an abnormal one.

O. T. SCHULTZ.

ATYPICAL LYMPHOGRANULOMATOSIS. M. A. SKWORZOFF and E. W. USSANOWA, Virchows Arch. f. path. Anat. **294**:595, 1935.

The authors report three cases, all in children, of involvement of the lymphoid system in which some of the nodes revealed the characteristic histologic picture of lymphogranulomatosis but others a proliferative reaction that was not histologically characteristic. This leads them to a discussion of histologic specificity in general and to the conclusion that histologic specificity is a relative and not an absolute matter. The histologic reactions of tissues depend on a variety of factors.

O. T. SCHULTZ.

Pathologic Anatomy

SYPHILITIC AORTITIS IN CHILDHOOD AND YOUTH. R. F. NORRIS, Bull. Johns Hopkins Hosp. **57**:206, 1935.

Two cases of syphilitic aortitis are presented, one in a 9 year old girl and the other in a boy of 17. There is some evidence that the lesions were congenital.

These were the only cases suggestive of congenital syphilis of the aorta among 14,000 autopsies at the Johns Hopkins Hospital. The fact that syphilitic aortitis with involvement of the coronary ostia may be, in rare cases, a cause of sudden death in young persons has been emphasized. Gross myocardial necrosis and scarring resulting in stenosis and atresia of the coronary orifices occur occasionally in cases of syphilitic aortitis and should be carefully searched for.

FROM THE AUTHOR'S SUMMARY.

THE GENESIS AND DEVELOPMENT OF BRUNN'S NESTS AND THEIR RELATION TO CYSTITIS CYSTICA, CYSTITIS GLANDULARIS, AND PRIMARY ADENO-CARCINOMA OF THE BLADDER. F. S. PATCH and L. J. RHEA, Canad. M. A. J. **33**:597, 1935.

The mucous membrane of the urinary bladder when exposed to chronic irritation may undergo metaplastic changes. The metaplastic changes may result in leukoplakia, cell nests of Brunn, cystitis cystica and cystitis glandularis. Epidermoid carcinoma may develop in the leukoplakic areas. Cysts which develop in the epithelial nests of Brunn are the result of an active secretory process rather than of a degenerative one. These cysts may develop into glandular formations lined with tall epithelial cells that can acquire the property of secreting mucus. The mucus-secreting epithelial cells that line the submucosal glands, as well as those present on the surface of exstrophic urinary bladders, are in the great majority of cases, if not in all, the result of metaplastic changes in the normal epithelium of the bladder, and are not derived from epithelial cells of the large intestine that have been misplaced during embryologic development. Cystitis cystica and, more especially, cystitis glandularis are potentially precancerous lesions, and from the latter mucus-secreting adenocarcinoma may develop.

FROM THE AUTHORS' CONCLUSIONS.

ANOMALOUS RELATIONSHIP OF THE RIGHT URETER TO THE VENA CAVA. A. RANDALL and E. W. CAMPBELL, J. Urol. **34**:565, 1935.

We have reported two cases of obstructive nephropathy with pyonephrosis caused by the passage of a ureter completely around the vena cava in its descent to the bony pelvis. The literature contains reports of three cases as found at autopsy and one as found at operation. To the latter group we now add our two observations.

FROM THE AUTHORS' SUMMARY.

THROMBOPHLEBITIS AND EMBOLISM. F. V. THEIS, Surg., Gynec. & Obst. **60**:996, 1935.

This study is of interest from a statistical standpoint. Of more than 14,000 injections (882 patients) for the obliteration of varicose veins, each one of which was given to produce an area of thrombophlebitis, only 1 was followed by pulmonary embolism, and this occurred after ten days' confinement of the patient to bed.

WARREN C. HUNTER.

INTRAMURAL GALLSTONE FORMATION IN THE GALLBLADDER. P. EISERTH, Centralbl. f. allg. Path. u. path. Anat. **63**:134, 1935.

Twelve linseed-sized gallstones were found in the wall of the gallbladder in the body of a woman 64 years old who died of senile marasmus. The gallbladder was enlarged and thin-walled. The stones protruded externally, leaving the lumen smooth, and were composed of lime salts and bile pigment. These stones occupied markedly enlarged Luschka ducts, in the lumen of which desquamative epithelium and mucus abounded. The muscularis about these ducts was atrophic but devoid of inflammation, and the ducts approached the serosa. The explanation offered for this condition is as follows: Stasis in the gallbladder increased the pressure

in it and resulted in marked elongation of Luschka's ducts. In these outpouchings desquamative epithelium and mucus collected and formed a nucleus for gallstones, with the bile pigment and calcium being added from the bile of the gallbladder. No reason is offered for the condition of the muscle. GEORGE RUKSTINAT.

FINER MORPHOLOGY AND DEVELOPMENT OF THE GLIA OF THE BRAIN OF THE NEW-BORN INFANT. H. N. ROBACK and H. J. SCHERER, Virchows Arch. f. path. Anat. **294**:365, 1935.

Forty-nine pages with twenty-seven illustrations are devoted to a presentation of the results of a study of the glia of the brain at and shortly before birth and of the development of the glia following birth. The purpose of the study was to furnish a morphologic basis for investigation of pathologic changes in the glia during this early period of life. The technical procedure elected was the Nissl method applied to large pyroxylin (celloidin)-embedded sections, usually frontal sections of an entire hemisphere. It is held that this method will yield the best results in studies of pathologic brains. The material consisted of fifty brains, twenty-four from infants from 46 cm. in length to 2 months of age, fourteen from infants from 2 to 13 months of age, the remainder from premature infants from 29 to 46 cm. long. The region of the internal capsule and corpus striatum was most intensively studied. The observations in these regions are compared with those in other parts of the brain. The most striking differences between the brain of the new-born and that of the adult are the presence in the former of areas of glial growth or germinal centers, situated chiefly in the neighborhood of the ventricles, and a seemingly chaotic intermingling of areas of glia in various stages of development. The latter state was found to be associated with an orderly sequence in the development of different portions of the brain, glial development going hand in hand with myelinization. With myelinization of the various tracts there occurs in these regions an active proliferation of the glia, a process to which the authors apply the term "myelinization gliosis." Myelinization requires the participation of the glia, and it is during this stage that the glia differentiates into macrodendroglia and oligodendroglia. O. T. SCHULTZ.

Microbiology and Parasitology

ANAPHYLAXIS IN THE ALBINO RAT WITH REFERENCE TO DIET AND HISTAMINE. H. N. PRATT, J. Immunol. **29**:301, 1935.

Forty-two albino rats were sensitized with horse serum or with egg albumin, and forty of them responded to injections administered after an interval of from six to fifteen days with anaphylactic shock of varying severity. The effects of different factors, including diet, sex, weight of body and weight of adrenals on the occurrence and intensity of anaphylactic shock were evaluated. The smaller and more malnourished the animals were, the more pronounced was the shock. The larger sensitizing doses were more effective with regard to both sensitization and production of precipitins. Contrary to previous reports, there was no relation between a bread diet or the weight of the adrenals and the incidence or severity of the shock. The pathologic changes in the shocked animals were distinct and particularly pronounced in the small intestines. The Dale technic proved not suitable for the demonstration of sensitization in the rat, but it helped to bring out the differences in the effect of varying concentrations of histamine on the tonus of the excised uterus. I. DAVIDSOHN.

THE CHEMICAL NATURE OF SHWARTZMAN-ACTIVE SUBSTANCES. K. APITZ, J. Immunol. **29**:343, 1935.

Substances which produce the Shwartzman phenomenon are contained in two different chemical fractions of bacteria. The so-called nucleoprotein fraction is

always found capable of producing the Shwartzman phenomenon; it is derived from bacterial autolysis or by extraction. Its potency is increased in an alkaline solution. Most of the potency of agar washings is connected with an alcohol-precipitable biuret-negative fraction. The appearance of these substances concerned in the Shwartzman phenomenon does not depend on autolytic but on metabolic processes. Those of the last-described fraction are highly unstable, precipitable with small volumes of alcohol and not identical with type-specific carbohydrates. That of filtered washings is slowly dialyzable and resists digestion by trypsin.

FROM THE AUTHOR'S SUMMARY.

SCARLET FEVER IMMUNIZATION OF NURSES. G. W. ANDERSON and W. I. REINHARDT, J. Infect. Dis. **57**:136, 1935.

The Dick test as carried on under a wide variety of conditions by many persons and with the solutions commercially available is a reliable index of immunity to scarlet fever. Five injections of Dick toxin conferred an immunity comparable with that indicated by a negative Dick test. This immunity lasts at least three years. Contact with scarlet fever does not increase the hazard of contracting the disease provided the subject has been immunized with the Dick toxin. Although the principal drawback in the use of the toxin is the severity of the reaction that it occasionally produces, this is never serious and does not constitute an excuse for failure to immunize those unduly exposed to the disease. Hospitals can virtually eliminate scarlet fever in their nursing staffs through routine immunization of all nurses found by the Dick test to be susceptible.

FROM THE AUTHORS' CONCLUSIONS.

THE SPECIFICNESS OF THE NEGATIVE PHASE IN PRECIPITIN PRODUCTION. L. HEKTOEN and W. H. WELKER, J. Infect. Dis. **57**:337, 1935.

The negative phase in precipitin formation appears to be specific. In rabbits immunized with a single antigen the injection of heterologous antigens has not reduced the precipitin content of the blood. In a rabbit immunized against many antigens the injection of one of the antigens resulted as a rule in disappearance from the blood of the precipitin for that antigen only. These results indicate that in the rabbit different precipitins exist as separate entities.

FROM THE AUTHORS' SUMMARY.

A PATHOGENIC DIPHTHEROID BACILLUS FROM MENINGITIS. H. J. GIBSON, J. Path. & Bact. **41**:239, 1935.

A fatal case of leptomeningitis associated with a diphtheroid bacillus of unusual characters is described. A study has been made of its morphologic, cultural and biochemical characters and of its pathogenicity. It is highly virulent for rabbits, guinea-pigs, rats and mice, and its effect is not neutralized by the antitoxins of Corynebacterium diphtheriae and Corynebacterium ovis. Efforts to demonstrate an exotoxin have failed, and indirect evidence has been presented which suggests that the pathogenic effects are not due to toxic action.

FROM THE AUTHOR'S SUMMARY.

CYTOLOGICAL EXAMINATION OF MILK FOR TUBERCLE BACILLI. S. T. COWAN and L. MADDOCKS, J. Path. & Bact. **41**:373, 1935.

A method of making films for a cytologic examination of milk is described. Several varieties of cellular aggregation are seen in tuberculous and nontuberculous milk, but of these only three types of endothelioid cell groups appear to be of importance. The presence of endothelioid cell groups in a milk sample is not evidence of tuberculous infection, but it is thought to be indicative of some form of mastitis. The examination of endothelioid cell groups facilitates the demonstra-

tion of acid-fast bacilli in films of milk, and in a series of 229 samples of unmixed milk 21 of 38 tuberculous samples (55.3 per cent) were detected by this means. The demonstration of acid-fast bacilli in an endothelioid cell group is considered to have much greater diagnostic significance than their demonstration without relation to the cytology of the film. No false positive results were obtained in this series, and in our opinion a report can be issued with confidence that Mycobacterium tuberculosis was present.

FROM THE AUTHORS' SUMMARY.

THE ESSENTIAL NEUROTROPISM OF THE YELLOW FEVER VIRUS. G. M. FINDLAY and R. O. STERN, J. Path. & Bact. **41**:431, 1935.

Viscerotropic yellow fever virus instilled into the nasal passages of rhesus monkeys produces ordinary yellow fever but inoculated by the same route into mice gives rise to encephalomyelitis. Rhesus monkeys inoculated intracerebrally with viscerotropic yellow fever virus after subcutaneous injection of yellow fever immune serum present encephalitic symptoms associated with microglial proliferation, perivascular infiltration and intranuclear inclusions in the brain and only slight lesions in the liver. Rhesus monkeys inoculated intracerebrally with viscerotropic yellow fever virus before the subcutaneous injection of yellow fever immune serum do not present encephalitic symptoms but die of ordinary yellow fever, the only lesion in the brain being microglial proliferation. In rhesus monkeys that die of encephalitis as a result of an intracerebral inoculation of viscerotropic virus, virus is often found at death only in the brain and not in the blood and liver, where it has apparently been neutralized by the subcutaneous injection of immune serum.

FROM THE AUTHORS' CONCLUSIONS.

THE SEROLOGICAL RELATIONSHIPS OF B. DIPHTHERIAE. J. F. MURRAY, J. Path. & Bact. **41**:439, 1935.

It is shown that while the gravis, mitis and "intermediate" types of Bacillus diphtheriae are serologically distinct from one another numerous subgroups exist within each type.

FROM THE AUTHOR'S SUMMARY.

REVERSED PASSIVE ANAPHYLAXIS IN THE GUINEA-PIG. C. E. KELLETT, J. Path. & Bact. **41**:479, 1935.

The injection of rabbit precipitating antiserum as antibody and horse antiserum as antigen into a guinea-pig previously treated with the homologous antigen (horse serum) will, under certain conditions, produce acute anaphylactic shock, a state of reversed passive anaphylaxis having been induced. If these conditions are to be satisfied an incubation period of forty-five minutes or more should elapse between the two injections, the dose of antigen must be an optimum one, and the dose of antiserum must be large, probably at least ten times that required to produce normal lethal passive sensitization, and it must be given by a route such as the intravenous one. Failure to satisfy certain of these conditions appears to explain adequately the negative results hitherto reported.

FROM THE AUTHOR'S SUMMARY.

PRESENCE OF HEMOLYTIC AND OTHER STREPTOCOCCI ON HUMAN SKIN. L. COLEBROOK, W. R. MAXTED and A. M. JOHNS, J. Path. & Bact. **41**:521, 1935.

Hemolytic streptococci of the kind usually associated with human puerperal infections (Lancefield's group A) were not found on the perineal and perianal skin of 160 women attending an antenatal department, and the risk of such streptococci's being conveyed to the genital tract from the feces is considered to be remote. Group A hemolytic streptococci were isolated from the hands of 7 of 181 normal persons (3.8 per cent). It seems probable that they were derived from the respiratory tract. Treatment of the mother's hands during labor by an antiseptic

is advocated. Nonhemolytic types of streptococci (chiefly Str. viridans) were found on nearly all the hands investigated but not on the skin of the interscapular region.

From the Authors' Summary.

Aetiology of Acute Rheumatism. B. Schlesinger and others, Lancet **1**:1145, 1935.

The deposits obtained by high speed centrifugation of pericardial fluid from persons with acute rheumatic pericarditis contain particles which morphologically resemble the elementary bodies of virus. Similar bodies have been found in the deposit obtained from one pleural exudate in association with rheumatic pneumonia. Relatively pure suspensions of these bodies in physiologic solution of sodium chloride containing 0.25 per cent of solution of formaldehyde (formalin) have been prepared. These suspensions are specifically agglutinated by the serums of patients who are suffering from, and successfully resisting, an acute rheumatic infection. The serums of patients in whom the infection is quiescent fail as a rule to agglutinate these suspensions. Completely negative reactions were also obtained with the serums of normal persons and with those of patients suffering from various nonrheumatic infections. On the evidence summarized it is suggested that the bodies found in pericardial exudates represent the infective agent of acute rheumatism. The importance of streptococcic infection as a factor in the causation of the disease is recognized. It is suggested that the lowered resistance produced by such infections enables the virus to enter the body or, if the virus is already lying latent in the tissues, allows it to assume active characters.

From the Authors' Summary.

Bovine Pulmonary Tuberculosis in Man. F. Tobiesen, K. A. Jensen and H. C. A. Lassen, Tubercle **16**:385, 1935.

The report presented includes twenty-six cases of bovine pulmonary tuberculosis, all in Copenhagen (1931-1933). Ten of the patients were under 5 years, none over 32 years, of age. Only in one of the cases could infectious tuberculosis be demonstrated in the home. Thirteen of the twenty-six patients had been drinking raw milk for some time, while only three patients denied having taken raw milk. Cervical adenitis was ascertained prior to or coincident with the demonstration of the pulmonary disease in eleven (perhaps thirteen) of the cases. The pulmonary processes appeared on roentgen examination not to show any particular features that would differentiate them from the changes characteristic of pulmonary tuberculosis of the respective age classes as generally seen. In eighteen of the twenty-six cases the tubercle bacilli were cultivated from material obtained by gastric lavage alone. Six of the patients died within the period of observation, and all six died of generalized tuberculosis; three were under 5 years of age, two between 5 and 15 years, and one over 15 years. It seems that the prognosis in bovine pulmonary tuberculosis in the age class under 5 years is serious, probably just as serious as that in pulmonary tuberculosis due to the human type of the bacillus.

H. J. Corper.

Serologic Differentiation of the Two Genotypes of Group A and Group B. P. Moureau, Sang **9**:484, 1935.

An attempt to differentiate the two genotypes of group A and B with the aid of the anti-O agglutinin present in ox serum was unsuccessful.

A. S. Wiener.

Histopathology of Experimental Postvaccinal Encephalitis. J. Sanz Ibáñez and A. P. Rodríguez Pérez, Trav. du lab. d. recherches biol. de l'Univ. de Madrid **29**:167, 1934.

Sixteen rabbits were killed twenty-four, forty-eight, seventy-two and ninety-six hours after intracerebral injections of the encephalic virus of Gallardo. As a

result of the study of the brains the following conclusions were reached: (1) An etiologic relationship exists between the vaccinal virus and the lesions of the central nervous system; (2) neuronal alterations of degenerative nature occur in the form of hyperchromemia and vascular degeneration; (3) the porencephalic cavities are due to the action of the virus; (4) perivascular infiltrations do not occur; (5) the neuroglia react with hypertrophy and gliosis; (6) the macroglia appear to migrate to the region of the lesion; (7) there is no specific microglial reaction due to the virus, the changes in these cells being due to necrosis of cerebral tissue; (8) the reaction of the virus is essentially ectodermal; (9) the myelin and oligodendroglia show no reaction to the virus, and (10) a meningeal reaction is interpreted as being a response to the virus. The article has an excellent bibliography.

CYRIL B. COURVILLE.

STRUCTURAL CHANGES IN THE CENTRAL NERVOUS SYSTEM FOLLOWING ANTIRABIC VACCINATIONS. A. TSCHERNJACHIWSKY and M. BIRKENHOF, Trav. du lab. d. recherches biol. de l'Univ. de Madrid **29**:263, 1934.

A 72 year old woman died about three weeks after having been bitten by a dog suspected of having rabies. After she had had twenty antirabic vaccinations weakness of the limbs developed, with retention of urine, headache and general lassitude. At autopsy, no Negri bodies were found in the hippocampus, but when dogs were given injections of material from different parts of the brain rabies developed. The nerve cells of the cerebral cortex showed a loss of Nissl's substance and irregularity and shrinking of the nucleus. Some of the cells presented characteristic sclerotic changes. The neurofibrils showed evidences of disintegration in Bielschowsky and Cajal preparations. The changes in the Purkinje cells were even more distinct. The neuroglia showed direct cell division, thought to be due to some stimulant resulting from necrosis of tissue. The perivascular feet of these cells were multiplied. Degeneration of the myelin sheaths, formation of corpora amylacea on the surface of the cerebral cortex and development of compound granular corpuscles were also present. Aside from the absence of Negri bodies, these observations are similar to those of rabic encephalitis.

CYRIL B. COURVILLE.

PERNICIOUS ANEMIA AND ACTIVE TUBERCULOSIS. E. MATHIAS, Frankfurt. Ztschr. f. Path. **46**:376, 1934.

The author comments on the fact that pernicious anemia, a wasting disease with marked cachexia, rarely is found simultaneously with active tuberculosis. It seems as if pernicious anemia creates immunity to the development of active tuberculosis. It may be possible that the protective mechanism lies in the reduction of the oxygen content of the red blood corpuscles in the lungs through diminution of their number. It is stressed that, as a result of the modern liver treatment, patients afflicted with pernicious anemia are no longer immune to active tuberculosis.

OTTO SAPHIR.

THE ORIGIN AND SIGNIFICANCE OF FUNGI IN THE CRYPTS OF THE TONSILS. G. SPONHOLZ, Frankfurt. Ztschr. f. Path. **46**:390, 1934.

The material on which this study is based was derived from 151 tonsils, 94 obtained surgically and 57 at autopsy. In 105 tonsils, fungi were present in surgical and autopsy specimens in about the same ratio. The fungi were found more commonly in hyperplastic tonsils from patients between 20 and 30 years old. Only very occasionally may fungi cause suppurative inflammation. However, they may lead to an accumulation of the material secreted by the neighboring salivary glands and possibly cause formation of sialoliths. The classification of the fungi meets with difficulty. They show some of the characteristics of trichomycetes and some of streptotriches.

OTTO SAPHIR.

THE ANTICOMPLEMENTARY ACTION OF RIPENED ORGANIC EXTRACTS. F. HAHN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:380, 1935.

Alcoholic extracts of beef heart kept at an elevated temperature are known to acquire anticomplementary properties. Hahn studied the effect of different factors on the development of the anticomplementary action. That oxidation is a factor was deduced from the interference of anaerobiosis with the appearance of anticomplementary changes. The proper degree of dispersion was another factor, as were also the method of dilution, the addition of cholesterol and the presence of electrolytes. Complete clearing of the extract removed its anticomplementary properties, though some degree of clearing was necessary. Hahn assumes that as a result of the factors named substances develop which react with the complement-bearing globulins of the guinea-pig's serum and so interfere with the function of the complement.

I. DAVIDSOHN.

THE IMMUNOLOGIC PROPERTIES OF THE STREPTOCOCCI FOUND IN THE MOUTH. T. ENDO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:410, 1935.

Streptococci were isolated from the saliva of thirty normal persons and from patients with dental caries, with pyorrhea and with alveolar abscesses. They could not be differentiated morphologically or biologically. By means of cross-absorption experiments with specific immune serums they proved to be related but could be divided into four distinct groups according to their origin (*a*) saliva, (*b*) caries, (*c*) alveolar abscesses and (*d*) pyorrhea. It was found that these streptococci were entirely different from the other known forms of streptococci.

I. DAVIDSOHN.

DOUBLE REGISTRATION IN THE SCHULTZ-DALE TEST. E. BÁRÁNY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:439, 1935.

Bárány found that by placing both horns of the uterus close to each other in the same water bath and by registering their contractions parallel to each other on the drum, the reliability of the Schultz-Dale technic was greatly increased, mainly owing to easier recognition of spontaneous contractions.

I. DAVIDSOHN.

THE VIRUS OF LYMPHOGRANULOMA INGUINALE. Y. MIYAGAWA, T. MITAMURA, H. YOAI, N. ISHII and J. OKANISHI, Jap. J. Exper. Med. **13**:723, 733 and 739, 1935.

Ultrafiltration of the Virus.—The virus passed through Chamberland L_2 and L_3, Berkefeld V and N, and Seitz E. K. filters. It passed through collodion membrane with pores larger than 0.33 micron but was retained when the pores were smaller than 0.24 micron. The part retained in the latter case was active and contained many special granule-like corpuscles.

Cultivation of the Virus on Chicken Chorio-Allantoic Membrane.—Some degree of successive cultivation of virulent material from the brains of inoculated mice was accomplished on the chorio-allantoic membrane of the chicken embryo. The granule-like corpuscles were found in the characteristic white plaque on the membrane.

Effect of Heat, Cold, Etc., on the Virus.—The virus was more resistant to cold than to heat; it also resisted desiccation to some degree; brain material was virulent in fairly high dilution. The special granule-like corpuscles found in material from persons with inguinal lymphogranuloma were also found in the experimental disease in mice and other animals. In guinea-pigs, human convalescent serum appeared to contain a virucidal substance.

STUDIES OF LYMPHOGRANULOMA INGUINALE FROM ETIOLOGIC AND IMMUNOLOGIC POINTS OF VIEW. E. WASSÉN, Acta path. et microbiol. Scandinav., supp. 23, 1935, p. 1.

The virus of inguinale lymphogranuloma may be transmitted by intracerebral inoculation from mouse to mouse without loss of virulence to man. It is also pathogenic in other animals. Typical lymphogranuloma developed in man from the intradermal and subcutaneous inoculation of virulent mouse brain. In these cases the Frei reaction developed within from six to thirteen days after the inoculation and at least in one case persisted for two hundred and eighty days. Between twelve and seventeen days after such inoculation the serum acquired the power to neutralize the virus for mice. Extracts from the organs of infected monkeys acted in the same way as the Frei antigen on intracutaneous injection in human beings.

Immunology

IMMUNITY WITH COEXISTING SENSITIZATION IN HAY FEVER. R. A. COOKE and OTHERS, J. Exper. Med. **62**:733, 1935.

Using the hay fever caused by ragweed as the representative of a certain type of allergy, we have made studies to determine the mechanism of the protection afforded by specific injections, thus far established only by clinical observation. Transfusions of blood and injections of serum from clinically immune, treated patients stopped the clinical reaction in untreated patients, thus indicating a transferable immunity. The amount of skin-sensitizing antibody in the serum was found to be practically unchanged by specific injections. Injection of allergen-antibody mixtures into normal skin showed an inmmediate (one hour) reaction where sites were made if serum of untreated patients (serum A) was used, but no reaction or a slight one if serum of treated ones (serum P) was used. When sites made with allergen-antibody mixtures were tested after forty-eight hours, reactions were absent with serum A mixtures if enough allergen had been used, but were positive with mixtures of serum P even though a much stronger allergen was contained in the mixture. The primary inhibition of reactions with mixtures including serum P was not due to the antihistamine effect, to binding of skin-sensitizing anitbody or to binding or lysis of allergen. The inhibiting antibody appears to be specific. These serologic studies supported by transfusion experiments have been interpreted by us as showing the development under treatment of a peculiar blocking or inhibiting type of immune antibody that prevented the action of allergen on the sensitizing antibody and hence showed in the type of human allergy under consideration (hay fever) the coexistence of sensitization and immunity.

FROM THE AUTHORS' SUMMARY.

CONVALESCENT SERUM IN PREPARALYTIC POLIOMYELITIS. M. BRODIE, J. Immunol. **28**:353, 1935.

Poliomyelitis is an entirely neurotropic disease. The invasion and spread of the virus occur along the nerve fibers. The febrile preparalytic stage must not be construed as indicating systemic invasion but is probably due to an early involvement of the midbrain. From these observations the conclusion is justified that the serum of convalescent patients and of normal adults has no place in the therapy of the disease. Relatively large doses of convalescent serum were administered by Brodie to six monkeys without apparent improvement, although in some of the animals the serum was administered at the earliest moment when the diagnosis could possibly be established and in one animal even earlier.

I. DAVIDSOHN.

THE RELATION OF HETEROPHILE IMMUNITY TO COLDS. GEORGE E. ROCKWELL and HERMAN C. VAN KIRK, J. Immunol. **28**:485, 1935.

A study of the blood of thirty patients who were treated with oral vaccine containing pneumococci and streptococci showed an inverse relation between the frequency of acute colds and the titer of heterophilic antibodies (antisheep hemolysins).

I. DAVIDSOHN.

GENETIC STUDIES OF THE AGGLUTINOGENS M AND N. H. S. HYMAN, J. Immunol. **29**:223, 1935.

The blood of 578 persons was studied for determination of the frequency of the types M, MN and N in the population of Columbus, Ohio. The inheritance of the properties A, B, M and N was investigated in 100 families with 220 children. The findings support the theory that M and N are inherited as a single pair of allelomorphic genes. It was found from the determination of the blood group properties in twenty pairs of identical twins and in two pairs of fraternal twins that blood grouping is one criterion for establishing the nonidentity of twins.

I. DAVIDSOHN.

THE RELATION OF HETEROPHILE ANTIGEN TO SERUM SICKNESS. H. M. POWELL, W. A. JAMIESON and G. F. KEMPF, J. Immunol. **29**:267, 1935.

To 10 volumes of a concentrated tetanus antitoxin was added 1 volume of a high-titered so-called heterophile immune serum that was produced by inoculating rabbits with a mixture of boiled erythrocytes of the sheep and cultures of Pneumococcus and of Bacterium lepisepticum. It was concluded that the heterophile (Forssman) antigen was removed by the treatment from the tetanus antitoxin because one rabbit did not respond with a production of heterophile antibodies to one injection of the treated antitoxin. Thirty-nine patients were given injections of the treated tetanus antitoxin, and serum disease developed in 30 per cent of them. The blood serum of five of the thirty-nine patients was studied for the presence of heterophile antibodies. Only one showed an elevated titer, and this patient did not have serum disease. From these results Powell and his associates conclude that the heterophile antigen which may be present in horse antiserums is not responsible for serum sickness.

I. DAVIDSOHN.

CHEMICAL STUDY OF THE POLLEN-SENSITIZING ANTIBODY COMBINATION. F. M. STEVENS, J. Immunol. **29**:273, 1935.

No differences were noted in the quantities of nitrogen in samples of ragweed pollen before and after immersion in serum of persons sensitive to ragweed. This indicated that nitrogen-containing antibody did not become attached to the ragweed antigen.

I. DAVIDSOHN.

Tumors

CARCINOMA AT EARLY AGES. L. WEISENSEE, Ztschr. f. Krebsforsch. **41**:1, 1934.

Thirty-four of 1,561 patients with carcinoma treated at the University Clinic in Wurzburg, Germany, during the years from 1919 to 1929 were less than 30 years of age. Their cancers were distributed as follows: breast, 0.8 per cent; stomach, 1.8 per cent; rectum, 2.2 per cent; skin, 2.6 per cent; esophagus, 2.8 per cent; colon, 3 per cent; tongue, 3.8 per cent; urinary bladder, 4 per cent, and oral cavity, 7.4 per cent. There were two cases of carcinoma of the kidney, one in a child of 1, the other in a child of 7, years of age. In general, these carcinomas in the young were of considerably greater malignancy, as evidenced by the shorter periods of survival, than were corresponding ones in older persons. The cancers of the skin showed, however, a reversed relationship. Nothing was observed to indicate that there was an increasingly frequent occurrence of juvenile cancer.

H. E. EGGERS.

NEW MORPHOLOGIC FINDINGS IN CARCINOMA. B. M. KLEIN and A. MISSRIEGLER, Ztschr. f. Krebsforsch. **41**:87, 1934.

By the use of a delicate and adequately described method of silver impregnation of absolutely fresh cancerous material, the authors demonstrate a finely fibrillar sheath about the normal cell which, with the development of malignancy, undergoes a series of changes culminating in an intracellular basket-like arrangement around the cell nucleus, to which they give the name *Panariocyte*. Intermediate changes similar to those in the progressive development of cancer are also present in benign tumors, but here the panariocytes do not occur. Similar changes have been observed in protozoa after suitable injury. The phenomena precede the appearance of changes in the cell demonstrable by more usual methods, and the authors consider them as the most delicate indicators of cellular injury. They believe that the demonstration of panariocytes may be of value in diagnosis, but only after the user has fully developed the rather delicate technic, and only with regard to the appearance of the culminating change.

H. E. EGGERS.

TOBACCO AND CANCER. O. SCHÜRCH and A. WINTERSTEIN, Ztschr. f. Krebsforsch. **42**:76, 1935.

Tobacco tar was found to be free from polycyclic, aromatic hydrocarbons. Painting the skin and mucous membranes of mice and rabbits with tobacco tar did not produce carcinoma even in conjunction with mechanical or thermic irritation. Fractions of tobacco tar likewise had no cancerigenic action. In rabbits subject to a high cholesterol diet and treated previously with coal tar, warts and carcinoma could be produced by means of tobacco tar. It seems that tobacco tar can be a factor in cancer only when predisposition exists.

FURTHER STUDIES ON THE AGENT OF CHICKEN LEUKOSIS. A. R. MEYER, J. ENGELBRETH-HOLM and E. UHL, Acta path. et microbiol. Scandinav. **12**:378, 1935.

The agent of leukosis can be bound by blood corpuscles from normal chickens and those that have spontaneously recovered, as well as by corpuscles from pigeons, rabbits, sheep and man. This binding is therefore regarded as a physical adsorption. The immunity resulting in the course of spontaneous recovery from leukosis protects the chicken not only against leukotic material of the strain with which the fowl was inoculated but also against material of other strains of leukosis, including our strain of combined leukosis and sarcoma. Correspondingly, naturally resistant animals are refractory to inoculation with material from pure leukosis strains and from the combined leukosis-sarcoma strains. Plasma from chickens that have spontaneously recovered is able to neutralize the free leukotic agent. Plasma from naturally resistant chickens appears to possess this inhibitory power too. The plasma from a chicken with slowly progressing erythroblastotic anemia was found to possess the power of inhibition, whereas no such property could be demonstrated in the plasma of an animal with acute leukosis.

FROM THE AUTHORS' SUMMARY.

Technical

COLLOIDAL GOLD REAGENT ACCORDING TO FOWWEATHER. W. HERRMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:279, 1935.

This is the second article by Herrmann portraying his experiences with the colloidal gold reagent of Fowweather for the study of cerebrospinal fluid. The reagent is prepared without heating, and the reduction of the gold salt is due to the action of sunlight. A double salt (gold potassium bromide) is used instead of the simple salt. By variation of the temperature of the fresh single distilled water and of the length of the exposure to sunlight the sensitiveness of the reagent can be adjusted at will. Herrmann recommends the preparation of the double salt

of gold and describes the technic in detail. The reagent proved to be very sensitive, stable and superior to all other known reagents for the colloidal gold test. It was found applicable for the diagnosis of syphilis, tuberculous meningitis and multiple sclerosis.

I. DAVIDSOHN.

TESTING AND ADJUSTING OF RESINS FOR THE COLLOID TESTS WITH CEREBROSPINAL FLUID. R. BRANDT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:425, 1935.

Brandt reported previously a procedure for testing and adjusting the reagent for the mastic test. In the present article, the applicability of the procedure for a large number of resins is reported. While all tested resins are in principle suitable for the preparation of a proper reagent, some of them are preferable to the others. The concentration of the alcoholic solution of the resin, the quantitative relations in the dilution with solutions of sodium chloride, the rapidity of the ripening process and the amount of alkali necessary to prevent flocculation by the diluent are among the factors which are analyzed.

I. DAVIDSOHN.

DRIED PREPARATIONS OF M AND N SUBSTANCES. B. JONSSON, Acta path. et microbiol. Scandinav. **12**:253, 1935.

The M and N receptors, like the A and B receptors, are located in the stroma of the red blood cells, few or none being present in the hemoglobin. Jonsson was therefore able to obtain dry preparations of M and N receptors by lysing red blood cells and removing the stroma by centrifugation. The stroma was washed with physiologic solution of sodium chloride and dried. This was a tedious process because it was difficult to remove the stroma by centrifugation. The procedure could be expedited somewhat by precipitating the stroma with acetone. The preparations thus obtained, though highly specific in absorption tests, were not very active because of their low solubility and the coarseness of the particles.

A. S. WIENER.

Society Transactions

PATHOLOGICAL SOCIETY OF EASTERN NEW YORK

ARTHUR W. WRIGHT, *Secretary*

Regular Meeting, Dec. 13, 1935

ELLIS KELLERT, *Presiding*

MYXOSARCOMA ARISING IN THE SUBCUTANEOUS TISSUE OF THE LEG. C. R. SMITH.

A white man aged 21 had had a small nodule beneath the skin of the upper and outer aspect of the left leg since childhood. Three months before he sought treatment, this nodule began to enlarge, and when first seen it measured 14 cm. in greatest diameter. The tumor was freely movable, not attached to skin or deep tissues. On palpation it was firm but not hard. A wide excision of skin was made and the tumor removed with the muscle sheath beneath.

It was an encapsulated tumor, 14 by 10 by 8 cm. in size. The skin over the outer aspect was thin. At the base of the mass there was a capsule, which the neoplasm appeared to invade. The sheath of the muscle was not involved. On section the tumor contained much gelatinous material. The glairy cut surfaces were separated into irregular lobules by thin septums of connective tissue.

Histologically the tumor was composed of irregular masses of myxomatous material separated by strands of dense fibrous connective tissue. The individual cells were stellate, and about them many fine interlacing fibrils were noted. Many of the cells were shrunken, and their nuclei appeared pyknotic. There was an occasional large cell containing one or more nuclei of irregular form. The growth extended into the capsule, but the muscle sheath showed no invasion. The tumor was considered to be a large, slowly growing myxosarcoma of the subcutaneous tissues. There was no recurrence three months after removal.

UNRECOGNIZED AMEBIC DYSENTERY IN A PATIENT WITH BRONCHOGENIC CARCINOMA. V. C. JACOBSEN.

A cigar factory foreman 54 years old had suffered from severe diarrhea for three days. Since 1920 he had been having intermittent attacks of bloody diarrhea associated with pains in the lower part of the abdomen. In 1922 proctoscopic examination showed a small area of bleeding 16 cm. above the sphincter. Gastro-intestinal roentgenograms revealed nothing. A diagnosis of mucous colitis was made. Stools were not examined for amebas. During the following eleven years he had frequent episodes of pain and bloody mucous diarrhea.

He was admitted to the hospital acutely ill, stuporous, rather dehydrated and greatly emaciated. The temperature was slightly elevated; the pulse and respiration rates were within normal limits. The abdomen was generally tender, with spasm in the epigastrium and nodular enlargement of the liver. The diarrhea was profuse, foul, bloody and mucoid. Amebas were not searched for in the stools. No organisms of the dysentery group were found on culture. A clinical diagnosis of carcinoma of the rectum was made. The patient steadily declined and died four days after admission.

At autopsy an ulcerating tumor mass, 2 by 1 by 1 cm. in size, was found in the bronchus of the upper lobe of the right lung. Microscopically this proved to be a primary bronchial carcinoma composed of spindle-shaped cells. The hilar

lymph nodes contained metastases. The mucosa of the entire ileum was ulcerated and congested, the ulcers in the upper portions being small and scattered and those in the lower portions numerous and large, also confluent and deep. The colonic mucosa was largely destroyed by extensive confluent ulcers. At a point 15 cm. from the sphincter there was a particularly large deep ulcer, 7 cm. in diameter, with high indurated edges, which suggested carcinoma. In the base of this ulcer were several perforations which gave rise to pelvic peritonitis. Microscopically all of these ulcers were typical of amebic infection, showing undermining of the mucosa and destruction of muscle layers. Amebas were abundant on the surfaces, in crypts and in the submucosa, where they invaded blood and lymph vessels. The amebas contained variable numbers of erythrocytes. The large ulcer in the sigmoid colon showed a hyperplastic glandular border and many amebas. The wall was perforated, with resulting acute peritonitis.

The liver weighed 2,525 Gm. and contained numerous secondary carcinomatous nodules. Microscopically the capillaries contained many tumor cells and an occasional ameba which had phagocytosed erythrocytes. The amebas were in various stages of degeneration. There was no evidence of inflammatory response to their presence.

ENDOMETRIOSIS OF THE SIGMOID COLON SIMULATING CARCINOMA. V. W. BERGSTROM.

An unmarried white woman aged 43 complained chiefly of menorrhagia, metrorrhagia and pain low down in the abdomen with, more recently, nausea, vomiting and marked anemia. There had been a profuse hemorrhage at each menstrual period for the past two years, and there was no history of constipation or of bleeding from the alimentary tract. Vaginal examination revealed a large soft cervix. The body of the uterus could not be felt. By rectal examination a mass about 5 cm. in diameter could be palpated as far up as the finger could reach. It suggested a fibroid tumor.

Panhysterectomy was performed. At operation a tumor-like lesion suggesting carcinoma was noted at the junction of the rectum and the sigmoid flexure. Because of the poor physical condition of the patient, this lesion was not touched. Eighteen days later, however, the sigmoid flexure and upper end of the rectum were resected.

On gross examination of the removed bowel carcinoma was strongly suggested, and on cross-section the walls were markedly hypertrophied and suggested linitis plastica.

Microscopic examination revealed an extreme degree of endometriosis of the wall of the bowel, extending from the mucosa to the serosa. The condition was so marked that in some fields viewed under low power magnification only endometrial tissue could be noted. Small amounts of blood were seen in some of the endometrial glands, but most of them were empty. Microscopic examination of the ovaries, removed at the first operation, showed multiple endometriomas, one of which had become a large chocolate cyst.

TUBERCULOSIS OF THE LIP. A. H. CURRY.

A man of 26 years who was apparently in good general health gave a history of having had, two years prior to his admission to the hospital, what he termed a "coldsore" on his lower lip. This lesion gradually enlarged and spread into the mouth, causing generalized pain and swelling of the affected lip. During these two years treatment included mouth washes and two applications of the roentgen rays. There were periodic remissions during which the swelling subsided but the ulcerated areas persisted. Three weeks prior to admission there was an acute exacerbation of the condition with some involvement of the upper lip.

The lips, especially the lower one, were swollen. The inner surface showed a spongy congested productive lesion which contained small fissures. The skin

of the face was normal. The cervical lymph nodes were not palpable, but the thyroid gland was somewhat larger than normal. The lungs revealed slight dulness at both apexes, slight accentuation of whispered voice sounds, and râles posteriorly which were best produced by coughing. The temperature was 99.7 F.

The Wassermann and Kahn reactions were negative. Dark-field examination of material obtained from some of the fissures showed no spirochetes. Streptothrix, Leptothrix, Actinomyces and pathogenic yeasts were not found on culture. Staphylococcus aureus was isolated.

Examination of frozen sections immediately after the removal of some of the diseased tissues gave the first clue to the diagnosis, small tubercles being found. Sections of tissue fixed in Zenker's fluid showed typical tubercles in which tubercle bacilli were demonstrated.

Roentgenograms of the lungs, made after the nature of the lesion was known, showed changes characteristic of acute and advanced pulmonary tuberculosis.

This case is reported because of the unusual location of the tuberculous lesion, the lack of typical clinical signs of tuberculosis, and the importance of pathologic study of excised tissue when conditions lend themselves to this procedure.

POLYPOID CARCINOMA OF THE FUNDUS OF THE STOMACH. ELLIS KELLERT.

A white man 54 years old had been anemic for a long time. About a year before he consulted a physician he began to experience sharp pains in the epigastric

Polypoid carcinoma of the stomach.

region, but there was no nausea, vomiting or loss of weight. On physical examination nothing abnormal was found except extreme anemia.

Occult blood was constantly present in the feces. A month before death the blood count, similar to previous ones, was as follows: erythrocytes, 1,700,000; leukocytes, 10,400; hemoglobin, 15 per cent (Newcomer); polymorphonuclears, 89 per cent; lymphocytes, 11 per cent. Anisocytosis, poikilocytosis, microcytes and macrocytes were present, but nucleated erythrocytes were not found. Achromia was marked, and platelets were normally abundant. The urine showed albumin and a persistently low specific gravity.

The patient refused to submit to a gastric test meal or a roentgenologic examination and died after several blood transfusions.

Necropsy revealed moderate ascites and marked bilateral hydrothorax. The heart, lung, spleen, pancreas and liver were comparatively normal on gross examination. Within the stomach there was found attached at the fundus a soft pale-brown cauliflower-like tumor mass, 10 cm. in diameter. The pedicle was fairly broad and situated at the greater curvature, where the serosa was dimpled. The growth was covered with a thin layer of grayish-yellow exudate. On section, the surface appeared grayish white and glandular. Sections showed numerous fully developed acini containing mucus or cellular débris. The acini invaded the muscle at the point of attachment and infiltrated the wall beneath the normal mucosa at the margin. The nuclei were large and hyperchromatic; mitotic figures, rare.

NEW YORK PATHOLOGICAL SOCIETY AND NEW YORK ACADEMY OF MEDICINE, SECTION OF MEDICINE

Joint Meeting, Feb. 18, 1936

N. Chandler Foot, *President, New York Pathological Society*

Paul Reznikoff, *Chairman, Section of Medicine, New York Academy of Medicine*

Milton Helpern, *Secretary, New York Pathological Society*

Clarence de La Chapelle, *Secretary, Section of Medicine, New York Academy of Medicine*

SYMPOSIUM ON ETIOLOGY OF NEOPLASMS

Hereditary Factors. Clara J. Lynch (by invitation).

In the investigation of the causes of cancer the evidence on the influence of heredity and that on the effect of external agents have seemed to come into direct conflict. Though both aspects of the problem have been supported by experiments on lower animals, the ease with which tar tumors are induced has made it seem as though heredity were of no importance. Certain observations which my colleagues and I have made in our laboratory on the susceptibility to tumors in mice reconcile these apparently contradictory points of view.

The observations described were made on primary tumors of the lungs similar to those described by Livingood, Tyzzer and others and on squamous cell carcinoma of the skin. That susceptibility to tumor is inherited is shown by three types of evidence:

1. Inbred strains of mice show marked differences in the incidence of spontaneous tumors of the lungs (for example, strain 1194 with 6.7 per cent and a derivative of the Bagg albino strain with 37 per cent in mice over 12 months old).

2. A cross between these strains indicated the segregation of mendelian factors. In the first hybrid generation the percentage of tumors (24.4 in mice over 6 months old) suggested that susceptibility is semidominant. When the hybrids were crossed back to the more susceptible stock the incidence in the resulting generation (32.2 per cent) was significantly higher than in the generation obtained from the back-cross to the less susceptible stock (7.3 per cent).

3. Among mice of mixed ancestry, the progeny from tumor-free parents showed a lower incidence of tumor of the lung than those from parents one or both of which had tumors.

That cutaneous tarring can cause cancer in mice was demonstrated by Tsutsui. By distributing the applications of tar to various areas Murphy and Sturm showed

that it is possible to induce a tumor in the lung without the complication of a tumor in the skin. Both methods were applied to the strains of mice in question.

The lung tumor technic increases the incidence of pulmonary tumors, though not equally in the two strains. The incidence increased from 6.7 to 22.4 per cent in the less susceptible strain and from 37 to 85.4 per cent in the more susceptible strain. In a cross between the strains the percentages of tar-induced tumors of the lungs again gave evidence of inheritance, the back-cross to the more and the less susceptible lines showing tumors in 81.1 and 39.5 per cent, respectively. The difference between these strains in susceptibility to tar-induced tumor of the lung has been maintained for from six to eleven generations.

Five lines of mice have shown marked differences in susceptibility to tar-induced tumor of the skin (the incidence varying from 0.0 to 73.6 per cent). Strains which are susceptible to tumor of the lung may or may not have a high incidence of tumor of the skin.

It is concluded that though external agents cause cancer in mice their action is limited by the constitutional type of the individual mouse; that differences in susceptibility are inherited; that susceptibility is organ-specific.

There are many examples of the influence of the environment in relation to human cancer. The evidence derived from the statistics of cancer, from rare types of human cancer and from tumors in identical twins has a bearing on the question of inheritance. The indications are that the environmental and hereditary factors, though both important, are not effective in the same ratio for all types of tumor. A fundamental knowledge of the nature of malignant disease will not be attained without an evaluation of the factor of heredity.

CHEMICAL FACTORS. FRANCIS CARTER WOOD.

The first to observe and report on neoplasms chemically produced in man was Percival Pott in 1775, who published records of a number of cases of cancer of the scrotum in chimney sweeps. It was not until 1922 that Passey showed that an ethereal extract of soot produced epithelioma of the skin when painted on albino mice, thus showing that the soot contained a soluble material which was carcinogenic. In recent years it has been shown that the laborers in certain divisions of the color industry have cancer of the bladder in great excess over the normal expectancy of the population not so employed. In the last twenty years great attention has been paid, chiefly in England, to the occurrence of cancer in those working in shale oil factories, in certain employees in the spinning industry, in briquette workers and in farmers who use soot as a fertilizer, a great excess of cancer being observable in all these classes of people. About twenty years ago the carcinogenic qualities of tars, oils and similar products began to be studied on a large scale by Yamagiwa and Ichikawa (1915), and Tsutsui (1918), by Kennaway and Twort in England and especially by Dreyfuss and Bloch in Switzerland. The latter showed that the carcinogenic agent in tar is a substance which distils at about 300 C. and forms an insoluble salt of tri-nitrophenol. About the same time Kennaway showed that any organic material when distilled would furnish carcinogenic material, and shortly afterward he proved that the carcinogenic substance was a hydrocarbon since it could be produced by the action of high temperatures on acetylene, C_2H_2; the polymerization products obtained by passing this gas through a hot tube showed carcinogenicity of a considerable degree. Mayneord and Hieger studied the fluorescent spectrum from a number of carcinogenic materials and found that it showed three bands in the near ultraviolet section at 4,000, 4,180 and 4,400 angstroms. This spectrum is also given by the anthracenes and a large number of their derivatives. Clar in Germany, working on the anthracenes, produced 1, 2, 5, 6-di-benzanthracene, a compound which is composed of five benzene rings arranged about a phenanthrene nucleus. Cook, who was studying the various products at the same time, tested di-benzanthracene and found it carcinogenic. He then proceeded to make a large number of other synthetic substitution compounds and produced some which retained a certain amount of

carcinogenicity, but in general the simpler the compound the more effective it was; that is, the substitution of methyl or ethyl groups in various portions of the di-benzanthracene molecule reduced its activity. Cook then synthesized di-benzpyrene, and after studying its properties he proceeded to isolate a few grams of this substance from 2 tons of coal tar. Di-benzpyrene is highly carcinogenic and is presumably the substance in the various coal tar distillates which acts to produce cancer in man.

Finally, Cook succeeded in producing a substance known as methylcholanthrene, which is also highly cancinogenic, beginning his synthesis with deoxycholeic acid, one of the bile acids which occur normally in the human body. He was led to this notion by the elucidation of the structure of cholesterol, which is a precursor of the bile acids, being particularly impressed by the presence of a phenanthrene group in the cholesterol molecule and the fact that some of the carcinogenic substances have been shown to possess certain resemblances to the female sex hormone, thus showing that a series of compounds of varied physiologic potencies contain the phenanthrene nucleus. Other types of aromatic compounds have been discovered which produce cancer, but they are usually of low carcinogenicity. It is interesting, in passing, to note that with the resolution of the structural formulas for the male and female sex hormones the male hormone differs only by the presence of a CH_3 group which the female does not possess. It is an interesting speculation to think how different the world might have been had Mussolini and Hitler lost their CH_3 groups before they started their political activities.

[Dr. Wood then showed a series of lantern slides demonstrating the extraordinary variety of tumors produced by the various synthetic carcinogenic substances —for instance, the early changes in the skin produced with a very dilute solution of di-benzpyrene: Within a month or so the growth of the cells downward into the corium suggests the early stages of epithelioma. Slides were shown demonstrating a combination of carcinoma and sarcoma of the breast of the rat and another series of tumors produced by a chemical of a nature as yet unknown, secreted by a parasite, including various types of osteogenic sarcoma with cartilage and bone induced in the livers of rats.]

Biologic Aspects. James B. Murphy (by invitation).

The discussion of the cause of cancer falls naturally under two headings. The first deals with the causal agents which are known to produce changes in the tissues which may lead to cancer. The second is concerned with the property acquired by the cell which enables it to multiply indefinitely. The inciters (or carcinogenic agents) include a variety of chemical, physical and biologic agents besides parasites, bacteria and viruses. Once the malignant process is started the inciters play no part in maintaining it. Experimentation on animals has shown that the inciters may affect almost all varieties of cell, but in cancer of man it is not possible in the majority of cases to determine just what the inciters are which start the process. The nature of the mechanism by which the cells under the influence of inciting agents acquire the ability to multiply indefinitely is not yet clearly understood. The preponderance of evidence from the study of transplanted and induced cancers as well as from the investigation of hereditary factors indicates that malignancy is a universal potentiality of cells and that its degree is determined by heredity. Cancer probably may be produced in any one with sufficiently prolonged exposure to an inciting agent, but the ease with which this is accomplished varies with the individual subject. The whole picture suggests that the essential factor is a breakdown in the intracellular balancing mechanism which normally controls growth and specialization of tissues. This possibly is a loss of the inhibitor or an accumulation of the stimulator. There is direct evidence to support this hypothesis. It is therefore considered that the malignant condition arises essentially in the cell itself and is not the result of something in the way of an infectious agent introduced from the outside.

DISCUSSION

HALSEY J. BAGG (by invitation): I have been particularly interested in certain phases of Dr. Lynch's work. In her studies of the behavior of certain pulmonary tumors, one particular question has often obtruded itself: How pure are the animal strains that one works with? I am sure that Dr. Lynch, for one, will be the first to acknowledge that the biologists find this a distressing problem. Nature has not treated the experimenters with animals as kindly as she has the chemists in this matter of the material they have to work with, but one does have an agent in the inbreeding of stocks that is of great aid in producing what are called relatively pure strains of animals. At best, however, they are but relatively homozygous when it comes to the study of cancer. May I briefly mention my own difficulties in a related field, that is, the inheritance of tumor of the mammary gland in mice? One would think that after ten or more years of inbreeding by sister-brother matings, generation after generation, the absence of spontaneous tumors of any kind would indicate that one had at last a relatively pure strain. I have found that the relatively simple experimental procedure of rapid breeding brought about by mating females soon after parturition and preventing the nursing of the young results in the production of mammary cancers even in so-called "nontumor" inbred strains, and here the action of a hormone from the ovary and the retention of irritating secretions within the mammary gland itself probably play important rôles. Can one use this procedure to obtain from a "low tumor" strain females which are still what one might call positive for the tendency toward tumors of the mammary gland? This my associates and I are trying to do. By such a method purer strains, either plus or minus, the tendency toward cancer may be produced, thus simplifying genetic studies; yet again one might find that there is no such thing as a strain without tendency toward tumor of the mammary gland. I wish I could suggest some such aid to the important and interesting experiments of Dr. Lynch on tumor of the lung in animals. In the work that she has described I feel a very important and significant point has been that the spontaneous and induced tumors that she described showed essentially the same mode of inheritance.

JACOB FURTH: The etiology of cancer has been considered from three different aspects—hereditary, chemical and biologic—but it is evident from the presentations of the three speakers that these various aspects of cancer are interrelated.

Heredity is now studied with consideration of chemical and biologic influences; e. g., the application of tar products increases the incidence in the lungs of tumors that are indistinguishable from spontaneous tumors of the lungs, and their occurrence depends on the genetic constitution of the animals (Murphy, Lynch). In a similar manner the occurrence of cancer of the breast can be increased by intense breeding (ovarian activity) (Loeb, Bagg and others).

Nor can the chemical aspects be studied without consideration of the biologic aspects. The estrus-producing hormone is a pure chemical; it stimulates the breast gland to hyperplasia and occasionally to neoplasia; it also stimulates connective tissue cells to the production of sarcoma at the site of subcutaneous introduction. A powerful cancerogenic chemical isolated from tar, 1,2-benzpyrene, produces estrus.

The chemical aspects cannot be separated from the microbiologic aspects. In fowls painted with tar, tumors arise containing cancerogenic viruses (McIntosh). The rôle of viruses in the production of tumors has been excellently presented recently by Dr. Rous before the Harvey Society. I also refer to his lecture concerning the evidence that the agents producing tumors in chickens are viruses.

The three speakers agree that cancer is a local problem concerned with the action of carcinogenic substances at the site of their introduction or origin or in certain tissues for which they possess specific affinities. This is in contrast to the older point of view which emphasized a general susceptibility to cancer. A few years ago my co-workers and I irradiated mice over the entire body with massive doses of roentgen rays in the belief that the general resistance of the animals to

spontaneous neoplasms would thereby be lowered. In the irradiated animals neoplasms occurred, but they were present in the organs that were profoundly damaged by the roentgen rays. The neoplasms produced in the ovaries by roentgen rays can be traced to the anatomic changes produced by the rays. It is very unlikely that such tumors and several different neoplasms are produced by viruses.

Concerning the relation of experimentation on animals to the study of cancer in man it may be mentioned that many of the problems discussed tonight were suggested by observations in man, e. g., cancerogenesis by tar and by sunlight. The tumor-producing viruses, on the contrary, are species-specific, and a search for viruses producing tumors in man would involve experimentation on the human being. The virus of the papilloma occurring in the rabbit has been only recently discovered, and it is likely that there are many unknown tumor-producing viruses.

The vast amount of the recently acquired knowledge concerning the etiology of cancer is impressive and has contributed to the prophylaxis of humankind against cancer.

JAMES EWING: There will doubtless be unanimous approval if I express to the readers of these excellent papers the appreciation of this audience. It would be difficult for any one person to attempt to comment on these authoritative communications dealing with three major problems in cancer research.

Dr. Lynch describes one of her carefully controlled series of experiments, which demonstrates beyond doubt the decisive influence of heredity on the incidence of tumors in the lungs of mice. She then cautiously considers how far the experimental results in mice are reflected in clinical experience with man. I think her conclusions are sound. They are much more conservative than those of Little, Wainwright, Wassink and others, who believe that there exists an increased susceptibility to cancer in general as well as an increased organ and system susceptibility. In reviewing the literature on heredity in cancer I have been struck by the uncertainty regarding several major questions in genetics. It has apparently not yet been determined whether the tendency toward cancer is dominant or recessive, whether the influence is carried by the chromosomes alone or also by the cytoplasm, or how many factors are concerned. One often hears that cancer represents a form of mutation. Dr. L. W. Strong tells me that genetics is rapidly approaching a stage at which it will be possible to determine whether malignant transformation ever takes the form of a mutation. One has always assumed that the genetic constitution is immutable, but Dr. Bagg has shown this evening that by overfeeding and rapid breeding of cancer-free strains of mice every one of the females may be forced to produce cancer.

Dr. Wood's discussion of cancerogenic cyclic compounds brings a blaze of sunshine in the generally foggy atmosphere of cancer causation. Here one finds a series of definite chemical compounds, which may be produced synthetically, all capable of producing cancer or sarcoma indifferently in a relatively short time. Some of these compounds are chemically related to certain hormones normally active in the body and to vitamins, and they are closely related to bile acids and the cholesterols. It might appear that the investigators have arrived at a solution of the problem of the exciting agents in many important or even all forms of cancer. However, it is difficult to conceive that malignant cell growth is arrived at only through the action of cyclic compounds. Dr. Kennaway once expressed to me the feeling that these compounds were probably active in a definite but probably limited field only. It may be emphasized here that so far as is known the natural hormones alone are not capable of inducing cancer but require the entrance of secondary chemical factors. Experts in work with viruses think that this secondary factor is a living virus. The great variety of structures observed in the experimental tumors raises many new problems for the morphologist and perhaps provides a new method of investigation of the significance of such morphology. However, I think the experienced tumor morphologist need not be stampeded by the new data.

Coming to Dr. Murphy's communication, one enters again the obscure realms of the problem of cancer, and even his close thinking and logical analysis do not

succeed in simplifying the matter. I am one of those who believe that the secret all wish to unravel is enshrouded in the very complex processes of cell physiology and cannot at present be relieved of obscurities. Dr. Murphy has formulated the doctrine that normal growth results from a nice balance between growth-stimulating and growth-inhibiting forces and that malignant growth occurs when there is a lack of inhibitors. He and his associates have accumulated much experimental evidence in support of this doctrine. I regard it as the most advanced and rational theory of malignant growth at present available, and much more substantial than many other theories now being put forward. It has also the great advantage of being susceptible of experimental investigation or proof. He modestly describes it as a working hypothesis, but I think its pursuit is likely to prove more profitable than that of the simpler chemical hypotheses. As he points out, the results with cancerogenic chemical agents show how the cancer process may be started but throw no light on the nature of the process after it has been started. All signs point to the conclusion that many factors are concerned in malignant transformations and malignant growth, and in this sense one must believe that the outbreak of cancer is conditioned rather than caused.

If the conditions leading to cancer and the process itself are complex and varied, one must probably look to clinical medicine and general pathology to elucidate them. The program of the evening seems to be deficient in the absence of any speaker from the clinical side; the alert clinician has an important part to play in the study of cancer causation. Schinz and Buschke, at the close of an elaborate review of the subject of the heredity of cancer, reach the conclusion that the real significance of heredity in cancer will have to be decided by clinical medicine. Perhaps other branches of the subject may have to pass the same test.

Book Reviews

Localized Rarefying Conditions of Bone as Exemplified by Legg-Perthes' Disease, Osgood-Schlatter's Disease, Kümmell's Disease and Related Conditions. By E. S. J. King, M.D., D.Sc., M.S. (Melb.); F.R.C.S. (Eng.); F.R.A.C.S., Honorary Surgeon to Out-Patients, Melbourne Hospital; Stewart Lecturer in Pathology, University of Melbourne. Price, $7.50. Pp. 400, with 70 illustrations (mainly roentgenograms). Baltimore: William Wood & Company, 1935.

This excellent monograph is divided into four sections. The first deals mainly with bone as a tissue and with its general pathology. In addition it briefly describes the following: (*a*) some generalized diseases, such as rickets, infantile scurvy and congenital syphilis, that affect growing bone; (*b*) the roentgenographic manifestations relating to various types of pathologic change in bone; (*c*) the time of appearance of the centers of ossification in the epiphyses of the tubular, vertebral and short bones and of the time of fusion of the epiphyses with the shafts, and (*d*) the blood supply and ligaments of the short bones so far as these may possibly bear some relation to the diseases considered in the rest of the book. The 54 pages devoted by the author to a discussion of bone as a tissue and to some features of its general pathology show that he recognizes the importance of such a background for the rest of the book. It is to be regretted, however, that the value of the treatment of this phase of the subject is so heavily obscured by a lack of simplicity and logical sequence.

The second section (almost half of the book) is devoted to a discussion of osteochondritis juvenilis. King gives a systematic and well documented exposition of osteochondritis juvenilis as it occurs in the various regions of the body. The condition in each locality receives full treatment as a separate clinical entity and has its own large list of references. For instance, the chapter on osteochondritis of the upper femoral epiphysis (Legg-Perthes' disease) is followed by 426 titles; that on osteochondritis of the tarsal scaphoid (Köhler's disease), by 166, and that on osteochondritis of the tuberosity of the tibia (Osgood-Schlatter's disease), by 170. Furthermore, not only have reports in the literature been collected, but the author attempts a critical evaluation of them, and this attempt already makes the monograph much more than a mere annotated bibliography.

King is inclined to accept the view of Sundt and others that osteochondritis juvenilis in its various locations may arise from different causes and hence vary in its pathologic features. The reviewer feels, however, that in view of the scarcity of pertinent pathologic specimens and the defectiveness of most of those hitherto available for study pathologists should not yet commit themselves on this point.

The third section (about 80 pages) treats of posttraumatic rarefaction of bone. This part, too, represents an admirable and largely successful effort to organize and to evaluate critically the large mass of often confusing material and argument which has accumulated in this field. This chapter again shows the deficiencies in the present knowledge which arise through lack of sufficient pertinent pathologic data. The author is on his guard against attempts which have been made to compensate for this lack by deducing the pathologic changes theoretically from the roentgenographic findings.

The reviewer appreciates the difficulties encountered by the author in his effort to classify the posttraumatic rarefactions. There is a certain amount of justification for dividing them, as King does, into a diffuse form (exemplified in osteoporosis of the bones of the hand after a wrench of the wrist) and a circumscribed form (illustrated in Preiser's disease of the carpal scaphoid and in Kienböck's disease of the semilunar bone). This division distinguishes the rarefactions, at least in

regard to their extensiveness. It avoids, however, the problem of etiology and pathogenesis. Thus there is still the question: Is necrosis of bone, followed by resorption and rarefaction of the necrotic bone, the basic change that occurs in the bones (for instance, in the bones of the hand in consequence of a wrench of the wrist), or is the rarefaction merely the result of an increased blood supply due to vasomotor disturbance, as Leriche supposes? Surely, Kienböck's disease and Preiser's disease frequently follow aseptic necrosis of the semilunar or scaphoid bones, with or without fracture.

The fourth section of the monograph consists of a chapter of 20 pages on osteochondritis dissecans. It has a bibliography of 169 titles and maintains the high standards of the major portion of the book.

The roentgenographic illustrations are both numerous and excellent. All portray conditions observed in the author's own experience. In showing the mass of clinical and roentgenographic literature which has already accumulated this book should discourage the mere repetitious reporting of cases of Perthes' disease, Osgood-Schlatter's disease, Kümmel's disease and other conditions. To the pathologist the work should be an incitement to the supplementation of roentgenographic findings by more detailed and careful study of such pathologic specimens as become available. Such study is more urgently needed than are experimental attempts to reproduce these conditions in animals.

Altogether, this book deserves wide circulation as a real contribution to the medical literature on the subjects of which it treats. Indeed, it is probably the most ambitious and at the same time the most succinct and comprehensive discussion of them at present available in the English language. It represents a type of monograph which is more and more acutely needed as hope fades of bringing the full range of the work that has been done on certain topics into the scope of one person's experience.

The Patient and the Weather: Volume I, Part 1: The Footprint of Asclepius. By William F. Petersen, M.D. Price, $3.75. Pp. 127, with 94 illustrations. Ann Arbor, Mich.: Edwards Brothers, Inc., 1935.

This relatively brief introductory part of Volume I of Petersen's series of monographs "The patient and the Weather" was preceded in publication by volumes II and III, which have already been reviewed (ARCH. PATH. **20**:504, 1935). On the fly-leaf are the opening sentences of Hippocrates' "Airs, Waters, Places." The hippocratic admonition to him "who wishes to pursue properly the science of medicine" that he "ought to consider what effects each season of the year can produce" forms the central theme of Petersen's presentation.

The preface is a somewhat satiric critique of present-day medical teaching and its tendency to lose sight of the patient in generalized contemplation of his disease, to fail to see the trees that make up the forest. That this tendency exists must be admitted, but mankind is probably better off for the rationalization of medical science that has occurred through it, although the individual patient may occasionally be the victim of some rather poor medical attention. The introductory first chapter continues the discussion, with a plea for a return to the hippocratic method of direct observation of the individual. Chapter II consists of quotations from the writings of Hippocrates, with comments by the author that emphasize Hippocrates' deep knowledge of the effects of the meteorologic environment on man. It seems like carrying the apotheosis of the "Father of Medicine" a bit far to interpret his statement that "when a man draws breath into himself the air first reaches the brain" as indicative of Hippocrates' knowledge of "the importance of proper oxygenation of the tissues of the brain" or the observation that "when . . . the urine is turbid . . . there either is or will be a headache" as "certainly definite recognition of the alkalosis."

The hippocratic foundation for his observations having been laid, Petersen returns from Greece to the United States. In Chapter III he traces the tracks of the cyclonic air movements from the northwest across the northern part of our country to the northeast. In the three following chapters he correlates the

meteorologic environment brought about by this "changing restlessness of the winds" with racial differentiation, defective physical development, defects of the special sense organs, malformations and cerebral capacity and with a group of diseases including endocrine disturbances, pernicious anemia, leukemia, gastric ulcer, cardiovascular-renal disease, epilepsy, cancer, syphilis, tuberculosis and acute infections. The statistical material for this study is derived chiefly from the government publication, "Defects Found in Drafted Men." The material is presented in the form of maps, "maps dealing with the human beings that populate the land, maps that show why they are what they are and why they are becoming more so."

Petersen states that he was tempted to use as the title of this opening monograph the term "Hippocratic America." He selected instead "The Footprint of Asclepius" as being "more picturesque." The latter title requires the acceptance of Aesculapius not merely as the god of the healing art but as god of the atmosphere. This leads into the field of controversial mythology, on which the author briefly touches. Some right to meteorologic supremacy one may accord Aesculapius through heredity, since he was the son of Apollo, god of the air, and through personal experience, since Zeus slew him with a bolt of lightning because of his proficiency in the healing of mortal man. The "big shot" of all the gods feared that through the skill of Aesculapius man might achieve immortality and so "muscle in" on the olympian theocratic "racket." In the maps of the United States that profusely illustrate the volume the deeper shades have been used for the most marked effects of the meteorologic environment. In this way there has been impressed on our country Aesculapius' footprint, the heel of which lies over the northwest Pacific states, the arch over the northern states to the west of the Great Lakes region, the broad ball of the foot over the latter region and the toes over the states of the northeast Atlantic seaboard. This pedal sign is imprinted on the title page of each volume.

The literary style is delightful and is not marred by the evidences of perhaps too hasty preparation that marked the second and third volumes. Reading what the author has to say against the background of a revivified Hippocrates is not a dry-as-dust task but a pleasant fireside diversion.

Post Mortems and Morbid Anatomy. By Theodore Shennan, M.D., F.R.C.S. (Edin.); Professor of Pathology in the University of Aberdeen. Third edition. Price, $9. Pp. 716, with 241 illustrations. Baltimore: William Wood & Company, 1935.

The first edition of this book was published in 1912 and the second in 1927. In the third edition many important sections have been rewritten and the remainder brought up to date. The first three chapters deal in a thorough manner with the general aspects of postmortem examinations. Then comes the systematic consideration of the morbid anatomy of the various organs and structures, following closely the order in which postmortems usually are conducted. A special chapter is given to postmortem examinations in poisoning and another to medicolegal reports and postmortem examinations of infants and of patients who have died while under anesthesia. Methods for preparing museum specimens and microscopic sections are described in an appendix of twenty pages, which also contains tables of "equivalent imperial and metrical measurements."

The descriptions of morbid anatomic changes and the directions for guidance in the examinations are clear, concise and competent. Only the briefest mention is made of the microscopic alterations, and all the figures illustrate the gross appearances. The illustrations, all in black and white, are commendable and will be helpful in practical work. Italics are used rather freely; Latin names are italicized, without, however, any capital initials. Occasional references to the literature are inserted in the text, frequently without mention of the author's name. In accord with British usage, Hodgkin's disease is considered under the name of lymphadenoma. There are hardly any lapses from descriptive objectivity and remarkably few omissions. The statement on page 115 that "in subacute endo-

carditis immune substances are developed in the blood and kill the bacteria in the circulating emboli, so that infarcts of the organs . . . do not as a rule suppurate" most likely does not tell the true story. Somehow the lesions of granuloma inguinale escape consideration. It is now known that this infection, may cause, in addition to lesions in the groins, grave changes and strictures of the rectum. In connection with myeloma no mention is made of Bence-Jones proteinuria. The peculiarities and omissions mentioned do not detract in any serious way from the practical value of the book. Medical students, assistants, pathologists in training, medicolegal examiners and physicians have here a valuable guide in postmortem work and in the study and diagnosis of morbid anatomic changes. Shennan's book will serve well now the same helpful purpose that Orth's "Pathologisch-anatomische Diagnostik" did in its day.

Bacteriology in Relation to Clinical Medicine Theoretical and Applied. For Students, Laboratory Workers and Practitioners in Medicine and Public Health. By M. N. De, M.B., M.R.C.P. (Lond.), Professor of Pathology, Medical College of Bengal, Calcutta; Bacteriologist to the Government of Bengal; and K. D. Chatterjee, M.B., Medical Registrar, Medical College Hospitals, Calcutta; formerly Research Assistant, Department of Pathology, Medical College of Bengal, Calcutta. Price, 30 shillings. Pp. 599, with 276 illustrations. Calcutta, India: "Statesman" Press, 1935.

The aim and scope of this book are indicated well by its title. Some of its distinctive features are: the complete exclusion of protozoology, parasitology and mycology; modern (American) nomenclature side by side with the familiar current terminology; numerous illustrations, mostly original and generally praiseworthy; more attention to the structural changes due to bacterial infections than is customary in textbooks of bacteriology, and a clear, orderly, precise and almost schematic presentation, with emphasis on topics of special interest to students and practitioners in tropical regions. The book contains a great mass of information and instruction for students of medicine and public health but useful also for practitioners and laboratory workers. According to the foreword by R. Knowles, the press work is better than usual in Indian publications. The book is recommended as a good guide to the study of bacteriology in relation to bacterial diseases in man and their diagnosis.

Books Received

THE SPECIFICITY OF SEROLOGICAL REACTIONS. Karl Landsteiner, M.D., the Rockefeller Institute for Medical Research, New York. Price, $4. Pp. 178. Springfield, Ill.: Charles C. Thomas, Publisher, 1936.

REPORT OF THE MEDICAL RESEARCH COUNCIL FOR THE YEAR 1934-1935. Presented by the Lord President of the Council to Parliament by Command of His Majesty, January 1936. Price, 3 shillings. Pp. 183. London: His Majesty's Stationery Office, 1936.

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF HEARING: III. THE LOCALIZATION OF SOUND. H. E. O. James. Medical Research Council Special Report Series, no. 207. Price, 9 pence. Pp. 38. London: His Majesty's Stationery Office, 1936.

ETUDES EXÉRIMENTALES RÉCENTES SUR LES MALADIES INFECTIEUSES. Jean Troisier, Professeur agrégé de Pathologie expérimentale et comparée à la Faculté de Médecine de Paris, Médecin de l'hôpital Beaujon. Price, 45 francs. Pp. 280, with 50 illustrations. Paris: Masson & Cie, 1935.

LE FONCTIONNMENT DU REIN MALADE. DIURÈSE—ALBUMINURIE—OEDÈME—GLYCOSURIE—CLASSIFICATION DES NÉPHROPATHIES—RECHERCHES EXPÉRIMENTALES ET CLINIQUES. Paul Govaerts, Professeur de clinique médicale à l'Université de Bruxelles. Price, 25 francs. Pp. 214. Paris: Masson & Cie, 1936.

MEDICAL MYCOLOGY. FUNGOUS DISEASES OF MEN AND OTHER MAMMALS. Carroll William Dodge, Ph.D., Mycologist, Missouri Botanical Garden; Professor, Henry Shaw School of Botany, Washington University, St. Louis. Price, $10. Pp. 900, with 142 illustrations. St. Louis: C. V. Mosby Company, 1935.

MEDICAL PAPERS. DEDICATED TO HENRY ASBURY CHRISTIAN, PHYSICIAN AND TEACHER. FROM HIS PRESENT AND PAST ASSOCIATES AND HOUSE OFFICERS AT PETER BENT BRIGHAM HOSPITAL, BOSTON, MASSACHUSETTS. IN HONOR OF HIS SIXTIETH BIRTHDAY, February 17, 1936. Price, $10. Cloth. Pp. 1,000, illustrated. Baltimore: Waverly Press, 1936.